DISTRIBUTION SPATIALE DES COMMUNAUTÉS DE VERS DE TERRE ET LEUR EFFET SUR LES GAZ À EFFET DE SERRE EN CHAMPS AGRICOLES ET EN BANDES RIVERAINE FORESTIÈRES

par

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Mémoire présenté au Département de biologie en vue
de l'obtention du grade de maître ès sciences (M.Sc.)

FACULTÉ DES SCIENCES
UNIVERSITÉ DE SHERBROOKE

Sherbrooke, Québec, Canada, mars 2020

Le 17 mars 2020

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SUMMARY

This thesis reports the findings from a research project that aimed to determine the effect of earthworms on greenhouse gas (GHG) emissions in forested riparian buffer strips (FRBS). This project had two research questions. Firstly, we wanted to determine how earthworms are distributed in agricultural ecosystems and whether they had a preference for FRBS over adjacent agricultural fields. Secondly, we wanted to determine the effect of earthworms on the emission of the three most prominent GHG (CO_2, N_2O and CH_4) and how the effect of earthworms is affected by soil characteristics, namely, soil origin and soil texture. We expected earthworms to have a preference for FRBS and for them to have a positive effect on GHG emissions.

For the first research question, we conducted a field survey on agricultural fields with adjacent FRBS in Southern Quebec and Ontario as well as in the Czech Republic. At each site, we quantified earthworm numbers from each functional group (anecic, endogeic and epigeic) and characterized the site by noting the percentage coverage of the different types of vegetation and analysing soil's physicochemical properties. We found that for Eastern Canada, earthworm numbers, organic matter and soil moisture were all higher in FRBS than in adjacent agricultural fields. However, in Czech Republic, earthworm numbers were higher in agricultural fields than FRBS and there was no significant difference in moisture between agricultural fields and FRBS. This indicated that moisture is an important variable in predicting the distribution of earthworms. Furthermore, we found that earthworm numbers are positively associated with organic matter, pH, clay content and the percent coverage of deciduous trees and negatively associated with sand content and the percent coverage of coniferous trees. Following these results, the next step was to determine what effect earthworms have on GHG emissions.

In order to determine the effect of earthworms on GHG emissions we conducted controlled microcosm experiments. These experiments were conducted using a replicated factorial design

comprising of 3 soil origins (deciduous FRBS, coniferous FRBS, agricultural field) x 2 soil textures (field conditions, high clay) x 3 earthworm life habits (anecic, endogeic, no earthworm). Soils originating from FRBS emitted more CO_2 than soils from agricultural fields with soils from deciduous stands having higher emissions than soils from coniferous stands. Soils with a higher clay content emitted less CO_2 than soils with a lower clay content. Soils with earthworms emitted more CO_2 than soils without earthworms, however, this effect diminished with time and was no longer significant after ten weeks. Additionally, soils with earthworms emitted more N_2O than soils without earthworms. For CH_4, the transformation rates were higher for soils from FRBS than soils from agricultural fields under both anaerobic and aerobic conditions.

With earthworms having an overall positive effect on GHG emissions, FRBS should be designed such that they prevent the establishment of earthworms. Therefore, coniferous trees would be preferable over deciduous trees. Firstly, earthworm numbers were shown to be negatively associated with coniferous tree coverage, and, in the event that earthworms do become established, GHG emissions were shown to be lower from coniferous soils than deciduous stands.

Key words: Greenhouse gas emissions, forested riparian buffer strips, earthworms

SOMMAIRE

Cette mémoire rapporte sur un projet de recherche qui visait à déterminer l'effet des vers de terre sur les gaz à effet de serre en bandes riveraines forestières. Cela consistait de deux questions de recherche. Premièrement, nous voulions déterminer comment les vers de terre sont distribués en milieux agricoles et s'ils ont une préférence pour les bandes riveraines forestières comparé aux champs agricoles adjacents. Deuxièmement, nous voulions savoir l'effet des vers de terre sur les principaux gaz à effet de serre (CO_2, N_2O et CH_4) et comment cela interagit avec les caractéristiques de sols, notamment, la texture du sol et la provenance, soit sous une plantation de feuillus, conifères ou un champ agricole. Nous prévoyons que les vers de terre auront une préférence pour les bandes riveraines forestières et qu'ils auront un effet positif sur les émissions des gaz à effet de serre.

Pour la première question de recherche, nous avons échantillonné des champs agricoles ayant des bandes riveraines forestières adjacentes situées à travers du sud de l'Ontario et du Québec ainsi qu'en République Tchèque. À chaque site nous avons quantifié le nombre de vers de terre de chaque groupe fonctionnel (endogé, epigé et anécique) et avons caractérisé le site en notant le pourcentage de recouvrement des strates végétales et en analysant un échantillon de sols pour des caractéristiques physico-chimiques. Nous avons trouvé que l'abondance des vers de terres et l'humidité et le pourcentage de matière organique du sol étaient plus élevés en bande riveraines forestières qu'en champs agricoles au Canada. Par contre, en République Tchèque l'abondance des vers de terre était plus élevée dans les champs agricoles et il n'y avait pas de différence d'humidité entre les sols des bandes riveraines et ceux des champs agricoles. Cela indique que l'humidité est très importante dans la détermination de la distribution des vers de terres. De plus, nous avons déterminé que le nombre de vers de terre est positivement associé au pourcentage de matière organique, le pH et le pourcentage d'argile du sol, le pourcentage de recouvrement des arbres feuillus et est négativement associé au pourcentage de sable du sol et le pourcentage de

recouvrement des conifères. Suivant ces conclusions, nous devions déterminer leur effet sur les gaz à effet de serre.

Pour la deuxième question de recherche, nous avons complété deux expériences en microcosmes pour déterminer l'effet des vers de terre sur les émissions de gaz à effet de serre. Ces expériences avaient 18 traitements comprenant une série factorielle de trois provenances de sols (bande riveraine feuillu, bande riveraine conifère et champ agricole), deux textures de sols (argile élevé et argile bas) et trois niveaux de vers de terre (anécique, endogé et aucun). Les sols de bandes riveraines ont émis plus de CO_2 que les sols de champs agricoles avec les sols feuillus ayant des émissions plus élevées que les sols conifères. Les sols avec l'argile élevé ont émis moins de CO_2 que les sols avec moins d'argile. Les sols avec des vers de terres ont émis plus du CO_2 que les sols sans vers de terres, mais cet effet a diminué avec le temps et n'était plus significatif après dix semaines. Les sols avec des vers de terres ont aussi émis plus de N_2O que les sols avec aucun vers de terre. Pour le CH_4, l'origine du sol était le facteur le plus important avec les sols de bandes riveraines ayant des taux de transformation de CH_4 plus élevés que les sols agricoles sous conditions aérobiques et anaérobiques.

Étant donné que les vers de terre augmentent les émissions de gaz à effet de serre, des bandes riveraines comprenant des conifères au lieu des feuillus serait préférable pour éviter l'établissement des vers de terres. De plus, les émissions de gaz à effet de serre sont moins élevées dans les sols de conifères alors l'effet des vers de terre serait plus basse s'ils réussissent à coloniser les bandes riveraines.

Mots clés : Gaz à effet de serre, bande riveraine forestière, ver de terre

ACKNOWLEDGEMENTS

I would first like to thank my supervisor Professor Robert Bradley of the Biology Department at Université de Sherbrooke. Throughout this project he allowed this paper to be my own work while guiding me in the right direction as I needed it. I would also like to thank my co-supervisor, Professor Joann Whalen, as well as the members of my supervisory committee, Professor Bill Shipley and Professor Naresh Thevathasan, whose support throughout this project kept me on the right track.

I would like to thank Dr. Bill Parsons for his guidance on laboratory analyses, Dr. Agnieszka Józefowska for her instruction on identifying foreign earthworms, as well as Miloslav Šimek for the donation of this laboratory and time which made our work in the Czech Republic possible. I would also like to acknowledge the number of students who assisted with the field work component of this project: Petra Benetková, Gabriel Boilard, Anne Bolduc, Raphaëlle Dubois, Marika Caoutte, Brent Coleman and Laurence Guay.

This project would not have been possible without the financial support of Agriculture and Agri-Food Canada's Agricultural Greenhouse Gas Program (AGGP) and Centre Sève's AgroPhytoSciences scholarship program.

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FRBS: forested riparian buffer strips

GHG: greenhouse gas

CO_2: carbon dioxide

N_2O: nitrous oxide

CH_4: methane

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CHAPTER 1

GENERAL INTRODUCTION

Agricultural contaminants constitute the most important factor contributing to the degradation in water quality in the United States. These contaminants originate from a number of sources such as pesticides, nutrients and sediments (Maas *et al*., 1984; U.S. Department of Agriculture, 1985). Several approaches have been implemented to mitigate the negative impacts of agricultural practices. One such practice is the planting or preservation of forested riparian buffer strips (FRBS). These FRBS can be defined as streamside ecosystems that are managed with the aim of enhancing water quality by controlling nonpoint source pollution and protecting the stream environment (Lowrance *et al*., 1997). In practice, planting trees in riparian environments has been widely recognized as a means to improve stream habitats and water quality (Parkyn *et al*., 2005) due to their ability to moderate stream temperatures, reduce the input of sediments, provide sources of organic matter and stabilize the stream bank (Osborne and Kovacic, 1993). Furthermore, FRBS can be effective at intercepting and absorbing large amounts of nitrogen that would otherwise enter the adjacent water body and cause significant stress to the aquatic ecosystem (Mayer *et al*., 2007; Fortier *et al*., 2010).

While the focus tends to be on improvements to water quality, FRBS also provide a number of additional environmental benefits. Streams and the associated riparian habitats play a vital role in structuring vertebrate communities, making the design of FRBS a key wildlife management issue for human-impacted environments (Anderson et al., 2004). Riparian zones provide habitats for fish, plants and wildlife with FRBS serving as landscape corridors by connecting habitats (Palone and Todd, 1997; National Research Council, 2002). Furthermore, while the planting of FRBS may decrease crop land, there is the potential for economic returns as FRBS provide woody materials to farmers resulting in income from timber (Schultz et al., 1995). With

this wide array of environmental benefits, FRBS strips have become widespread in agroecosystems. However, in order to objectively asses the net environmental benefits of FRBS, we also need to identify the potential downsides of FRBS. One criterion of particular interest is the effect of FRBS on greenhouse gas (GHG) emissions. Namely, are they serving as a source or sink for GHG emissions?

The rising atmospheric concentrations of GHGs made the identification of potential GHG sources and sinks an area of great importance. In 2013, the concentration of atmospheric CO_2 surpassed 400 ppm for the first time in recorded history and current trends project that it will continue rising up to 1500 ppm (NASA, 2019). The concentration of CH_4 is increasing at a rate of approximately 2 % per year (Ramussen and Khalil, 1981), and, with CH_4 having 21 times the global warming potential of CO_2 (Nicks *et al*., 2003), this can lead to an additional 0.4 ºK increase in the earth's surface temperature over the next 40 – 50 years (Ramussen and Khalil, 1981; Wang *et al*., 1976). Since the 1800s, the relative concentration of N_2O to air has increased by almost 20 %. Over recent decades, the atmospheric concentration of N_2O has been increasing at a rate of approximately 0.25 % per year, and these trends are expected to continue (Wuebbles, 2009). This rise is of significant concern since N_2O has a warming potential 310 times greater than that of CO_2 (Nicks *et al*., 2003). With their rising concentrations and global warming potential, these three gases – CO_2, N_2O and CH_4 – can be thought of as the three most prominent GHGs, making them the focus of this study.

Soils can serve as a major source or sink for the three most prominent GHGs. Approximately 20 % of global CO_2 emissions originate from the soil (Rastogi and Pathak, 2002), as well as about one third of CH_4 and two thirds of N_2O emissions (Smith *et al*., 2003). Consequently, is assessing the rise in atmospheric GHG it will be necessary to study factors that will affect soil GHG emissions. These GHGs are released from the soil as a result of a number of biotic processes, namely respiration for CO_2, methanogenesis for CH_4 and a combination of nitrification and denitrification for N_2O (Wrage *et al*., 2001; Kool et al., 2010). Understanding

the factors that control these GHG producing processes will be of significant importance when assessing the ability of FRBS to limit soil GHG emissions. These factors include substrate availability (e.g. labile carbon or mineral nitrogen in the case of N_2O), and soil physico-chemical properties (e.g. pH and moisture), which will ultimately dictate microbial activity. Both substrate availability and soil physio-chemical properties may be significantly affected by earthworms (Lubbers *et al*., 2013). Consequently, the presence of earthworms is yet another factor that will need to be studied to determine the GHG balance of FRBS.

Earthworms have been deemed ecosystem engineers due to their ability to greatly modify the environments that they inhabit. For example, their feeding, burrowing and casting activities can change the soil structure, redistribute organic matter and alter the habitat of the microorganisms inhabiting the soil (Brown and Lavelle, 2000; Lavelle *et al*., 1997; Neilson and Hole, 1964). The resulting physical changes to the soil will influence processes at the ecosystem level, including: carbon storage, nitrogen transformation rates and the loss of nutrients via pore spaces (Bohlen *et al*., 2004). Additionally, these changes will affect the aforementioned regulatory process that control soil GHG emissions. As such, the presence of earthworms will be an essential component in studying the GHG balance in FRBS. Furthermore, the changes made by earthworms to the soil structure may negatively affect the ability of FRBS to perform environmental services. Although earthworms have been praised for improving soil structure and fertility (Fonte et al. 2019), both of these benefits are irrelevant in FRBS. Conversely, the burrowing activities of earthworms produce soil macropores, which create preferential flow pathways and increase the leaching of nutrients into adjacent streams (Schneider et al. 2017) thereby decreasing the ability of FRBS to sequester agricultural runoff. In order to propose an optimal FRBS design that minimizes the negative effects of earthworms, this study has been divided into two research questions.

Firstly, we need to determine how earthworms are distributed in agroecosystems. More specifically, are earthworms more abundant in FRBS than in adjacent agricultural fields? This

will involve determining which environmental characteristics drive the distribution of earthworms in order to predict earthworm abundance, and community composition, in both agricultural fields and FRBS. If earthworms are deemed to have a net negative effect on FRBS, then this data can be used to propose a design that will repel earthworms. Secondly, once we know how earthworms are distributed, we need to determine what effect they have on GHG emissions. Within the context of studying their effects in FRBS, we will need to determine how the earthworms' ability to affect GHG emissions interacts with the soils that they are inhabiting. More specifically, how does the earthworm effect interact with soil origin and soil texture?

Before separating these two questions, one element that will play an important role in both research questions is earthworm life habit. Earthworms are divided into three groups (anecic, endogeic and epigeic) based on their lifestyle. The differences among these three groups may lead to different selection criteria for optimal habitats, and will affect the degree and manner in which earthworms alter the soil. Anecic earthworms are the largest of the three groups in terms of biomass. They feed on litter from the surface which they incorporate into the soil using deep vertical burrows. Endogeic earthworms live in and feed on mineral soil and the associated organic matter. Epigeic earthworms live on the surface. They feed on fresh litter on the surface and do not make any permanent burrows (Edwards, 2004).

The differences among the earthworm groups highlight the necessity to study earthworm type in addition to earthworm abundance. For instance, the ecological effects of earthworms, and subsequently their effects on GHG production and emission, may be tied to their life habit. Both anecic and endogeic earthworms create burrows which will maintain soil porosity, drainage and aeration, which will affect the diffusion of GHGs from soils (Edwards, 2004). Additionally, these two groups may have a significant effect on overstory plant communities. Anecic earthworms act as ecosystem engineers by structuring the soil environment and incorporating large quantities of litter and seeds into the soil. This relationship with seed dispersal can influence the structure of plant communities (Eisenhauer *et al*., 2008). For endogeic

earthworms, they ingest seeds and modify them as they pass through the earthworm gut. This link with post seed dispersal predation indicates endogeic earthworms can have a strong impact on soil seed banks, and consequently drive plant community composition (Eisenhauer *et al*., 2009). Epigeic earthworms will directly affect the decomposition of the soil by ingesting, digesting and assimilating the organic matter and microorganisms of the soil, which are subsequently released in earthworm casts (Monroy *et al*., 2008).

For the first of our two research questions, we want to understand how earthworm communities are distributed in agroecosystems and whether there is preference for FRBS over adjacent agricultural fields. We expect FRBS to serve as a refuge for earthworms in agroecosystems. Firstly, FRBS would have fewer physical disturbances than adjacent agricultural fields. Secondly, in comparison to agricultural fields, FRBS are expected to have higher moisture and more organic matter as result of continuous leaf litter inputs. Therefore, FRBS are expected to be preferable environments for earthworms, which have shown to favour more humid soils with high organic matter (Edwards and Bohlen, 1992). Due to the preference of both anecic and endogeic earthworms for surface litter (Edwards, 2004), the preference for FRBS is expected to be stronger for these two earthworm groups in comparison to the soil feeding endogeic earthworms.

In determining whether FRBS are serving as a refuge for earthworm populations, we first need to study the effect of physical disturbances on earthworms. It has been widely regarded that conventional tillage has a negative impact on earthworm populations (Chan, 2001; Smith *et al*., 2008; St. Remy *et al*., 1989; Slater and Hopp, 1948; Barley, 1961; Low, 1972; Springett, 1992; Friend and Chan, 1995; Mele and Carter, 1999). Furthermore, earthworm populations are typically higher in undisturbed habitats than in cultivated lands (Edwards, 1983; Edwards, 1992; Fraser, 1986). Cultivation can have a significant effect on earthworm communities, particularly on species that make deep burrows. Cultivation produces mechanical damage which will destroy permanent burrows and expose earthworms to bird predators. A single cultivation is not thought

to have any drastic effects on earthworm populations, whereas repeated heavy cultivation will progressively lower earthworm populations (Edwards, 2004). However, Curry *et al*. (2002) found that earthworms can be virtually eliminated over a single season as a result of drastic forms of soil cultivation. Conversely, no till and conservation till practices favour the build up of larger earthworm populations, where the only limiting factor is food availability (Edwards and Bohlen, 1996). This supports the prediction that earthworm numbers would be higher in FRBS than in adjacent fields.

While energy yielding substrates in organic matter derived from soil are expected to be more limited in agricultural fields than in FRBS, the use of organic fertilizers can provide a readily available food source for earthworm populations. Edwards (2004) found that when agricultural lands receive organic wastes, earthworm populations may double or triple over the course of a single season. However, the same study also showed that the use of some liquid manures which have not aged or composted can have negative effects on earthworm populations when they are applied to the soil as slurries, but these effects tend to be short term. Furthermore, inorganic fertilizers can also indirectly raise earthworm numbers by increasing crop yields which increases the amount of crop residues added to the soil (Edwards, 2004). Although fertilizers may provide earthworms with an additional food source, ammonia-based fertilizers often have adverse effects on earthworm populations, especially when these fertilizers are applied annually over many growing seasons, because earthworms are very sensitive to ammonia due to changes to the soil's pH (Edwards and Lofty, 1982). These characteristics of intensive agriculture coincide with lower earthworm abundances and reinforce the prediction that earthworm communities will favour FRBS. However, in order to fully understand how earthworms are distributed in agroecosystems, we need to determine all the environmental characteristics that affect earthworm distribution. Subsequently, we can determine how the identified significant explanatory characteristics are distributed in agroecosystems. Ultimately, are the favourable environmental characteristics associated with FRBS or agricultural fields?

In addition to organic matter and moisture, several other factors may control earthworm numbers in agricultural lanscapes. For example, a positive relationship has been observed between pH and the abundance of both *Lumbricus. rubellus* and *Aporrectodea calignosa*. (Crumsey *et al*., 2014). Additionally, soil texture has the potential to affect earthworm distribution (Nuutinen *et al*., 1998; Baker & Whitby, 2003). Sand content, water holding capacity, and the interaction of these two variables have all shown to have a significant effect on earthworm abundances; with sand content having a negative effect and water holding capacity having a positive effect on earthworm abundances (Crumsey *et al*., 2014). For instance, Hendrix *et al*. (1992) found lower earthworm abundances in sandier soils. This relationship is likely due to the fact that coarse textured soils have a low capacity to hold water and organic matter, making them less favourable for earthworms (Lee, 1985). Conversely, soils that contain more fine particles, such as clays, would have higher soil organic carbon (Bruce *et al*., 1990) which would be more favourable for earthworms. However, the strongest soil texture relationship observed by Crumsey *et al*. (2014) was between silt content and earthworm biomass. A similar relationship was observed by Reynolds and Jordan (1975) and Owen and Galbraith (1989) who attributed it to better moisture, temperature, physical substrate, pH and organic matter in loamy soils.

The type of vegetation may indirectly affect earthworm distribution though changes to the soil properties. For instance, plant litter quality is known to affect several soil properties and ecosystem functions, including nutrient cycling (Freschet *et al*., 2013) and carbon storage at the ecosystem level (De Deyn *et al*., 2008). Additionally, plant litter quality will be a criterion of interest since earthworm abundances are not only influenced by food quantity, but by food quality as well (Lee, 1985; Edwards and Bohlen, 1996; Edwards, 2004). Therefore, in order to understand how the interactions between earthworm abundances and soil properties will affect the distribution of earthworms in agroecosystems; we need to look at the environmental factors that influence the underlying soil characteristics, including overstory vegetation.

Stand type will be of particular interest in studying the effect of overstory vegetation on soil properties and earthworm distribution. Determining whether coniferous or deciduous stands are ideal for earthworm populations will be essential in proposing optimal FRBS designs. Firstly, litter derived from coniferous trees tend to have a moderately acidic or acidic pH (Stevenson, 1994). As such, coniferous buffers would be less favourable to earthworms which were shown to have a positive relationship with pH. Another important property in earthworm distribution is organic matter. Prescott *et al*. (2000) found evidence that broadleaf litter decomposed more quickly than needle litter. This has been attributed to lower lignin content and higher N concentrations in broadleaf litter. Therefore, deciduous stands may have more readily available organic matter for earthworm populations and would likely provide a more favourable environment. For both these stand types, the closed canopy would provide more shading to the underlying soil than the adjacent, more exposed, agricultural field. This difference in shade is important because earthworm densities have shown to be influenced by soil temperature (Berry and Jordan, 2001; Wever *et al*., 2001; Baker and Whitby, 2003). Berry and Jordan (2001) found that temperatures above 25 ºC were fatal to *L. terrestris* after about 180 days, and fatalities occurred as soon as after 14 days of exposure to temperatures above 30 ºC. Similarly, Wever *et al*., 2001 found that optimal earthworm growth occurred in soil incubated at 15 and 20 ºC. The shaded FRBS would have comparatively cooler soils than the adjacent field, which would likely prevent soils from rising above the potentially fatal 25 ºC mark, making them favourable to earthworms. Additionally, the increased shade would prevent surface evaporation from the soil; contributing to the more favourable moisture conditions in the FRBS.

For our second research question, we want to determine how earthworms affect the soil emissions of the three most prominent GHGs (CO_2, N_2O and CH_4) in FRBS. Additionally, we want to determine how these effects differ among earthworm life habits. In the interest of proposing optimal FRBS designs, we will need to determine which tree species should be planted such that soil GHG emissions as affected by earthworms are minimized. Therefore, we will also study how the earthworm effect interacts with soil origin and soil texture. To begin,

we need to identify the mechanisms by which earthworms affect the production of CO_2, N_2O and CH_4.

For CO_2, earthworms can accelerate the initial phase of plant litter decomposition, which would increase the short-term emissions of CO_2 (Liu and Zou, 2002). Many studies (Contreras-Ramos *et al*., 2009; Speratti and Whalen, 2008; Binet *et al*., 1998; Butenschoen *et al*., 2009; Hedde *et al*., 2007; Aira *et al*., 2008) have reported that earthworms increased CO_2 emissions. However, it is important to note that this effect has shown to be, first and foremost, a short-term process, as each of the aforementioned studies had a relatively short experimental duration. A meta analysis by Lubbers *et al*. (2013) found that earthworm-induced CO_2 emissions decrease with the duration of the experiment and disappear completely when the experimental period surpasses 200 days. The disappearance of the earthworm effect over this time frame implies that while earthworms accelerate the initial decomposition of carbon, they may not be increasing the total amount that is decomposed over the long-term. This opposite effect that occurs over the long-term could be explained by the proposed ability of earthworms to stabilize carbon in the soil.

It has been suggested that earthworms promote long-term soil carbon stabilization by protecting carbon in microaggregates which are formed into large macroaggregates (Bossuyt and Hendrix, 2005; Pulleman *et al*., 2005). This led to the suggestion that earthworms promote soil carbon storage, thereby reducing net CO_2 emissions (Six *et al*., 2004). Conversely, the aforementioned review by Lubbers *et al*. (2013) found that earthworms did not increase soil organic carbon stocks, therefore, they did not stimulate carbon sequestration. However, the 200-day time frame that was available for this review is a very short period to detect carbon sequestration. Therefore, if earthworms do stimulate carbon sequestration, as has been suggested in the literature, it is likely due to their ability to change the stability of the soil organic carbon – for instance, through the physical protection of soil organic carbon which makes the stocks less susceptible to breakdown over the long-term (Bossuyt *et al*., 2005). As proposed by Fragoso *et al*. (1997),

earthworms may have opposite effects at different temporal scales. They argued that in a time frame of hours, days and weeks, earthworms will assimilate and decompose carbon. Contrarily, on the time scale of months to years, earthworms have shown to reduce the decomposition of carbon by physically protecting carbon in aging casts (Six *et al*., 2004).

For N_2O, production occurs primarily during a particular type of decomposition, denitrification, which requires anaerobic conditions. Additionally, N_2O can be produced through nitrification and/or nitrifier denitrification by ammonia-oxidizing bacteria. Both of these chemoautotrophic processes require partly anaerobic conditions (Kool *et al*., 2010). With respect to earthworms, the conditions in the earthworm gut are ideal for denitrifying bacteria because it provides an anaerobic microsite with a continuous source of labile carbon and nitrogen, as well as moisture levels which promote denitrifier activity (Drake and Horn, 2006). These optimal N_2O production conditions extend beyond the earthworm gut and include all earthworm made structures, including: casts, burrow walls and mucus. Consequently, emissions from burrow walls have been as high as three times greater than emissions from bulk soil (Elliott *et al*., 1991). However, in general, the effect of earthworms on N_2O emissions is often small but stable, and tends to peak after the application of crop residues or organic fertilizers (Velthof *et al*., 2002). Furthermore, earthworms generally only cause a measurable increase in N_2O emissions over longer periods of time: i.e., exceeding 30 days (Giannopoulos *et al*., 2010; Rizhiya *et al*., 2007; Nebert *et al*., 2011). This will have the highest effect on net emissions if earthworm numbers increase in FRBS.

For both N_2O and CO_2, earthworms also influence soil emissions through indirect processes. Earthworms incorporate plant residues and mix the soil which stimulates soil aggregation and changes soil moisture dynamics, as well as gas diffusivity (Chapuis-Lardy *et al*., 2010; Giannopoulos *et al*., 2010; Lubbers *et al*., 2011; Rizhiya *et al*., 2007).

With CH_4, there has been less of a consensus as to whether earthworms have a net positive or net negative effect. Some studies have reported a positive effect, such as Koubová *et al*. (2002) who found that earthworms increase net CH_4 production and Borken *et al*. (2000) and Kamman *et al*. (2009) who found that earthworms decreased net CH_4 oxidation. Conversely, other studies have identified a negative effect where earthworms, or their structures, increased net CH_4 oxidation (Park *et al*., 2008; Moon *et al*., 2010; Kim *et al*., 2011). The effect of earthworms on CH_4 emissions is not a direct relationship, but rather the result of changes to the soil environment. That is, no CH_4 release has been detected from the earthworm gut (Karsten *et al*., 1997; Šustr and Šimek, 2009) and methanogens could not be isolated in the intestines of *L. rubellus* or *O. lacteum* (Karsten *et al*., 1997). One of the major mechanisms by which earthworms affect CH_4 emissions is through changes to the soil structure. Earthworms increase the aeration status of the soil. As such, soils with earthworms achieve greater CH_4 diffusion rates which will affect how much is converted to CO_2 prior to being emitted (Singer *et al*., 2000). Other studies have focused on the effect of earthworms on methanotrophs; for example, Park *et al*. (2008) found that amending landfill cover soils with earthworm casts increased the abundance of methanotrophs which stimulated the oxidation of CH_4. However, the direct effects of earthworms on methanogen communities remain unclear (Koubová *et al*., 2002).

One possible explanation for the lack of consensus on the effect of earthworms on CH_4 emissions could be the study of net emissions. The consumption and production of CH_4 is mediated by redox sensitive microbial processes (Yang and Silver, 2016). These processes can occur simultaneously and are controlled by microbial populations that are ecologically and evolutionarily different. Since net rates cannot distinguish between these two processes, they can mask significant the gross production and/or consumption of trace gases. Consequently, the failure to include gross rates in our models leads to inaccuracies that do not allow us to accurately predict how soil-atmosphere fluxes of CH_4 will respond to changes, such as the addition of earthworms, since the CH_4 oxidizing and reducing populations will respond differently to changes in environmental controls. On the other hand, gross production rates will look at the total CH_4 production (Zinder, 1993; Hanson and Hanson, 1996; King, 1997).

Therefore, in order to accurately assess the effect of earthworms on CH_4, one must study gross transformation rates.

Having outlined the proposed links between earthworms and the three most prominent GHGs, we now want to know how these effects interact with soil characteristics. Firstly, we want to know the effect of texture, more specifically, how increasing clay content affects GHG emissions. Secondly, we are interested in the effect of soil origin, which will allow us to determine which stand types (coniferous FRBS, deciduous FRBS, agricultural field) minimize soil GHG emissions as affected by earthworms and will allow us to propose optimal FRBS designs.

In comparison to silt and sand, clay particles have more pore spaces, meaning they will hold a greater volume of water under wetting conditions. Additionally, finer particles, such as clays, will have a greater surface area than coarser particles, such as sands. Since the forces that hold water to soil are surface-attractive forces, this means clays would have more adsorbed water than sandier soils. As such, clay enriched soils would hold more water for a longer period of time. The production of N_2O and CH_4 both require anaerobic conditions; therefore, due to the greater volume and longer retention of water, clay enriched soils are expected to produce more of these two gases. However, having more water-filled pore spaces would also decrease gas diffusivity which could lower soil emissions of the three GHGs from clay enriched soils. In addition to holding more water, the larger surface attractive forces of clays would also increase adhesion to organic matter. This would facilitate the formation of aggregates, and, since this the proposed mechanism by which earthworms stabilize carbon in soils, it is expected that earthworms inhabiting clay enriched soils would have lower net GHG emissions.

With CO_2 being the result of decomposition and N_2O and CH_4 production being limited by available carbon and/or nitrogen, the amount of organic matter in the soil will be an important

determinant in the soil GHG emissions. As previously outlined, FRBS are expected to have higher organic matter content than agricultural fields. Therefore, we would expect soils from FRBS to emit more GHGs than soils from agricultural fields. Additionally, the difference in the quality of organic matter between deciduous and coniferous stands has the potential to affect soil GHG emissions. Differences in the chemical composition of coniferous and deciduous litters will result in differences in the underlying soil. Soils dominated by deciduous trees tend to have higher carbon and nitrogen content and a slightly higher C:N ratio than coniferous soils (Rahman and Tsukamoto, 2013). Since the processes which produce GHGs are controlled by the substrate availability of labile carbon and mineral nitrogen (Lubbers *et al*., 2013), the higher soil nitrogen and carbon content of deciduous soils is expected to result in higher emissions than coniferous sands. Furthermore, conifer dominated stands tend to produce more acidic soils, which will decrease the rate of chemical processes such as nitrogen mineralization. An additional consideration is the higher lignin content in coniferous litter than deciduous litter. It has been repeatedly observed that higher lignin concentrations are correlated with slower rates of decay (Melillo *et al*., 1982; Harmon *et al*., 1990; Heim and Frey, 2004; Kurokawa and Nakashizuka, 2008). As such, the ability to speed up decomposition in coniferous soils would be limited in comparison to deciduous soils.

In order to determine optimal FRBS designs, a study which looks at the demography of earthworms in agricultural landscapes will be imperative. With these two research questions, we will be able to achieve our overall objective of proposing a FRBS design that minimize soil GHG emissions as affected by earthworms. A field survey will be used to quantify earthworm abundances, and community compositions, as explained by environmental variables. This will allow us to determine which type of landscapes are most likely to attract earthworm populations. Controlled microcosm experiments will be used to determine the effect of earthworms of GHG emissions, and how this effect interacts with soil characteristics. Once we determine whether earthworms have a positive or negative effect on GHG production, we will suggest a FRBS design that will deter or promote earthworm establishment respectively.

CHAPTER 2

THE DISTRIBUTION OF EARTHWORMS IN AGROECOSYSTEMS WITH FORESTED RIPARIAN BUFFER STRIPS AS EXPLAINED BY SOIL AND SITE CHARACTERISTCS

This chapter reports on a field survey that was conducted in Eastern Canada (Southern Quebec and Ontario) and Central Europe (Czech Republic). The aim of this study was to identify environmental variables that explain the distribution of earthworms in agroecosystems, with an emphasis on determining whether there is a preference for FRBS over adjacent agricultural fields. To do this, we collected data on earthworm abundances and community composition along with environmental data, including: soil physiochemical properties and over-story and under-story vegetation. Using multivariate analyses, we identified which environmental variables best explain differences among earthworm abundances and communities. This study brings a more comprehensive understanding of factors driving earthworm distribution to the literature. What separates this study from others of a similar nature is its scope. Our study consisted of a large number of sites across a large geographic range which allowed us to make conclusions that were not confounded to single location.

This project was completed with the help of a number of co-authors. Firstly, Robert Bradley was my supervisor and assisted throughout the study with the design, analyses and writing. My co-supervisor Joann Whalen assisted with the design of the field survey and provided perspective on how to begin looking at the data. Petra Benetková helped select and obtain permission for the sample sites in the Czech Republic. This work was also made possible with the assistance of Agnieszka Józefowska who showed us how to identify the earthworms from the Czech Republic at the species level as well as Brent Coleman and Naresh Thevathasan who helped with the work taking place in Southern Ontario.

Distribution of earthworms in agroecosystems with forested riparian buffer strips as explained by soil physiochemical properties and overstory vegetation

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2.0 Abstract

Forested riparian buffer strips (FRBS) are common in temperate agroecosystems due to their ability to sequester nutrients from agricultural runoff. The full environmental benefits of FRBS can only be evaluated, however, by accounting for a wide range of criteria that go beyond stream water quality. For example, the presence of earthworms which can modify the environments they inhabit through their feeding, burrowing and casting activities. We hypothesised that FRBS are a refuge for earthworms in agricultural landscapes due to higher moisture and litter inputs, and fewer physical disturbances. A field survey was conducted, in 2017 and 2018, to quantify earthworm species abundances in FRBS and adjacent agricultural fields in Eastern Canada and Central Europe. At 77 sites, we collected and identified earthworms, noted the tree species and understory vegetation in the FRBS, type of crop in the adjacent agricultural field, soil drainage class as well as five soil physicochemical variables (texture, pH, total C, total N and % organic matter). Earthworm abundance was significantly higher in FRBS than in adjacent fields for Easten Canada but higher in agricultural fields than FRBS for Central Europe. Distance-based redundancy analysis (dbRDA) revealed that the strongest positive correlation was between endogeic earthworm species and the percent coverage of understory vegetation, namely herbaceous and graminoid plants. Additionally, regression tree analysis for Eastern Canada

underscored the positive effect of clay content, moisture, treatment, organic matter and pH on earthworm numbers. Similarly, regression tree analysis for Central Europe highlighted the negative effect of treatment and sand content on earthworm numbers.

2.1 Introduction

Forested riparian buffer strips (FRBS) are increasingly prevalent in temperate agroecosystems due to their capacity to absorb nutrients from agricultural runoff (Fortier et al. 2015). FRBS may also provide habitat and migration corridors for wildlife (Palone and Todd 1998; Machtans et al. 1996), improve the ecological integrity of streams (Angermeier and Karr 1984; Bladon et al. 2016) as well as provide woody material to farmers resulting in additional income from timber (Schultz et al. 1995). Objectively assessing the net environmental benefits of FRBS requires, however, that we also identify potential downsides of planting trees in riparian zones. For example, FRBS may be a refuge for earthworms across agricultural landscapes. This prediction is based on the premise that soil moisture and plant litter inputs, both of which bolster earthworm survival and growth (Presley et al. 1996; Sileshi and Mafongoya 2007), are higher in FRBS than in adjacent agricultural fields where intensive plowing will decrease earthworm populations. Although earthworms have been hailed for improving soil structure and fertility (Fonte et al. 2019), both of these benefits are irrelevant in FRBS. On the other hand, the burrowing activities of earthworms produce soil macropores, which create preferential flow pathways and increase the leaching of nutrients into adjacent streams (Schneider et al. 2017). Furthermore, earthworms dwelling under temperate forest canopies may increase soil greenhouse gas (GHG) emissions such as N_2O (Fugère et al. 2017). For this reason, our first objective was to confirm that earthworm populations, soil moisture and soil organic matter were indeed greater in FRBS than in adjacent agricultural fields.

The degree and manner by which earthworms alter soil properties are dependent on their life habits, which are typically classified into three groups: anecic, endogeic and epigeic. Anecic species feed on surface litter, which they incorporate into the soil via deep vertical burrows. Anecic earthworms usually have a larger total biomass than earthworms in the other two groups (Edwards, 2004) and thus have a greater potential to increase soil nutrient leaching (van Schaik et al. 2014) and GHG emissions (Borken et al. 2000). For their part, endogeic species live exclusively belowground in the rooting zone, feeding on organic matter associated to mineral soil particles (Edwards 2004). To a lesser extent than anecic species, endogeic earthworms may also increase soil nutrient leaching by increasing soil porosity (Shipitalo and Bayon 2004), and they have also been shown to stimulate the production of N_2O (Augustenborg et al. 2012). Epigeic species, typically found under rocks and coarse woody debris, are surface dwellers that feed on fresh surface litter and do not make permanent burrows (Edwards 2004). Epigeic earthworms may increase nutrient leaching by accelerating the decomposition of organic forest floors (Hale et al. 2005), which can hold more water than mineral soil (Gupta and Larson 1979). Given the different environmental impacts of these three earthworm life habits, our second objective was to assess their relative abundance in FRBS compared to adjacent agricultural fields.

If earthworms in general or certain types of earthworms, do diminish the environmental benefits of FRBS, it would then be useful to design FRBS with characteristics that repel earthworms. This would first require that we understand how certain earthworm species or earthworm life habits correlate with specific soil and vegetation properties. For example, the low capacity of coarse textured soil to hold water and organic matter, as well as the abrasive nature of sand, may present an unfavorable habitat to earthworms (Hendrix et al., 1992). It is thus possible that soil dwelling earthworms (i.e. anecic and endogeic species) are negatively affected by high sand content, more so than epigeic species. Yet another example of what may control earthworm abundances in FRBS is the preference of different earthworm species for various food sources. For example, anecic species that process large quantities of fresh forest litter might be disadvantaged by acidic coniferous needles that contain more lignin than deciduous leaves.

Hence, our third objective was to explore earthworm distribution patterns in FRBS as a function of soil and vegetation characteristics.

According to Tiunov et al. (2006), comparing the distributions of Lumbricidae species across macro-scales may provide important insights into the potential of different earthworm species to spread into new habitats. For this reason, we conducted our survey of earthworms in FRBS and adjacent agricultural fields, in both Southeastern Canada and Central Europe. Considering their similar climates, edaphic conditions and land uses, we expected the factors shaping earthworm distributions in both of these bioregions to be similar. On the other hand, each bioregion holds a distinctive combination of attributes and is occupied by a particular assemblage of species that could interact with earthworm populations in a specific way. Furthermore, the spatial distribution of earthworm populations is not expected to be wholly determined by habitat. For example, earthworm invasion patterns previously observed in North America appeared to be strongly governed by ecologically neutral processes such as the regional species pool, whereas those in Europe were strongly governed by niche-based factors such as climate and life history traits (Tiunov et al. 2006). Hence, our fourth and final objective was to test the generality of earthworm distribution patterns in FRBS and adjacent agricultural fields, across a broad spatial scale.

2.2 Materials and Methods

2.2.1 Study sites

The field study was conducted in two bioregions (*sensu* Vilhena and Antonelli 2015), Eastern Canada (i.e. Southern Ontario and Southern Quebec) and Central Europe (South Bohemian Region of the Czech Republic). For Eastern Canada, we used ArcGIS (Environmental Systems

Research Institute (ESRI), Redlands, CA) and QGIS software (https://qgis.org/en/site/forusers/download.html) in order to select 60 sites with either corn (*Zea mays* L.) or soy (*Glycine max* (L.) Merr.) as the crop, and an adjacent forested riparian buffer strip of 10–100 m comprising either a deciduous, coniferous or mixedwood stand. For Central Europe, we used the LPIS online mapping tool (http://eagri.cz/public/app/lpisext/lpis/verejny2/plpis/) to select 17 sites based on the same criteria, with the exception that crops were either oats (*Avena sativa* L.) or cabbage (*Brassica oleracea* L.). Five of the sites in Eastern Canada and eight of the sites in Central Europe were managed meadows. From hereon, fields vs. FRBS will be referred to as "treatments".

2.2.2 Experimental design and field sampling

From May to August of 2017 and 2018 (Eastern Canada), and from October to November of 2018 (Czech Republic), a field survey was conducted to quantify earthworm abundance and to characterize soil and vegetation, in both the field and riparian buffer strip at each site. At each site, three quadrats (60 cm x 60 cm) located at least 20 m apart were dug to a depth of 30 cm, in both the field and riparian buffer strip. This topsoil from each quadrat was hand sifted to collect earthworms. Subsequently, we added 4 L of dry mustard solution (10 g L^{-1}) into the hole that was dug in each quadrat, to expel any deeply burrowing earthworms (Chan and Munro 2001). In the riparian buffer strips, rocks and coarse woody debris were lifted within a 150 m^2 circular plot established around each quadrat in order to collect surface-dwelling (i.e. epigeic) earthworms. Within these plots, we also noted the percent canopy cover of coniferous and deciduous trees, as well as the ground cover of shrubs, ferns, mosses, herbaceous and graminoid plants. Similar plots were established in the adjacent agricultural field to establish the percent cover of the various plant types. A topsoil sample (ca. 1 kg) from each quadrat was placed in a cooler and brought back to the laboratory for subsequent analyses (see below). Likewise, all earthworms collected at each site were fixed in 10% formaldehyde and brought back to laboratory for identification.

2.2.3 Soil analyses and earthworm identification

Gravimetric soil moisture content was determined by determining weight loss after drying fresh subsamples at 105 °C for 36 hours. Soil pH in water and in 1 M KCl solution was measured using a standard hydrogen electrode (soil:liquid = 1:2). Total C and N were determined by gas chromatography following high temperature combustion, using a Vario Macro CN Analyser (Elementar Gmbh, Hanau, Germany). Percent organic matter was determined by loss on ignition at 400 °C for 16 h. Soil texture was determined using the hydrometer method (Bouyoucos, 1962).

Earthworms collected in Eastern Canada were identified to the species level using Reynold's key (Reynolds 1992), whereas those collected in Central Europe were identified using keys developed by Csuzdi and Zicsi (2003) and Pižl (2002). Given that different earthworm species differ in size, earthworm abundances were also converted to biomass (Table 1).

Table 1. Ash-free dry mass estimates for earthworm species

Species Name	Ash-free dry mass (AFDM) conversion equation	Length (mm)	AFDM (g)
[1,2,3]*Allobophora chlorotica*[a]	[4]ln(AFDM) = 2.2853*ln(Length)-11.9047	[5]30-70	0.05158
[2,3]*Aporrectodea caligniosa*[b]	[4]ln(AFDM) = 2.2853*ln(Length)-11.9047	[5]60-85	0.12058
[1,2,3]*Aporrectodea longa*	[4]ln(AFDM) = 2.2853*ln(Length)-11.9047	[5]90-150	0.38142
[1,2,3]*Aporrectodea rosea*	[4]ln(AFDM) = 2.2853*ln(Length)-11.9047	[5]25-85	0.06414
[1,2,3]*Dendrobaena octaedra*	[4]ln(AFDM) = 2.2853*ln(Length)-11.9047	[5]17-60	0.02839
[1,2,3]*Eisenia foetida*[a]	[4]ln(AFDM) = 2.2853*ln(Length)-11.9047	[5]35-130	0.16200
[1,2,3]*Eiseniella tetraedra*[a]	[4]ln(AFDM) = 2.2853*ln(Length)-11.9047	[5]30-60	0.04054
[2,3]*Fitzingeria playura montana*[c]	[4]ln(AFDM) = 2.2853*ln(Length)-11.9047	[5]90-300	1.15683
[1,2,3]*Lumbricus castaneus*	[4]ln(AFDM) = 2.2853*ln(Length)-11.9047	[5]30-50	0.03098
[1,2,3]*Lumbricus festivus*	[4]ln(AFDM) = 2.2853*ln(Length)-11.9047	[5]48-105	0.13632
[1,2,3]*Lumbricus rubellus*	[4]ln(AFDM) = 2.2853*ln(Length)-11.9047	[5]50-150	0.25145
[1,2,3]*Lumbricus terrestris*	[4]ln(AFDM) = 2.2853*ln(Length)-11.9047	[5]90-300	1.15683
[1,2,3]*Octolasion cyaneum*	[4]ln(AFDM) = 2.6117*ln(Length)-13.0190	[5]65-180	0.59720
[2,3]*Octolasion lacteum*	[4]ln(AFDM) = 2.6117*ln(Length)-13.0190	[5]65-180	0.59720
[1,2,3]*Octolasion tyrtaeum*	[4]ln(AFDM) = 2.6117*ln(Length)-13.0190	[5]25-130	0.19064

[1]Reynolds, 1992; [2]Csuzdi and Zicsi, 2003, [3] Pižl, 2002; [4]Hale et al., 2004; [5]Reynolds, 1977. Notes: [a]Allometric equations not reported in Hale et al. (2004), used equation that represented all species excluding *Octolasion*. [b]*A. caligniosa* groups *A. trapezoides, A. tuberculata* and *A. turgida* from Reynolds (1992 and 1977). [c]Used values for *L. terrestris* when estimating AFDM.

2.2.4 Statistical analyses

All data statistical analyses were performed using R statistical software, version 3.4.1 (R Core Team, 2017). For each bioregion, we compared earthworm community structure in each treatment by performing MANOVA tests, using the number of individuals per life habit as the combined response variable and the identity of each site as a random effects variable. We then compared the relative abundance of each life habit in each treatment by performing t-tests. As there were significant differences in earthworm community structure in each bioregion and in

each treatment, we converted earthworm counts to biomass using published allometric equations for each species.

We used mixed model ANOVAs to test the effects of bioregion and treatment, as well as their interaction, on earthworm abundances and biomass, soil moisture and soil organic matter. These models also used site identity as a random effects variable. When significant interactions were found, we used *t*-tests to evaluate the effect of treatment on each response variable within each bioregion.

As there were proportionately more fields with meadows than with intensive agricultural crops in Central Europe than in Eastern Canada, we tested whether differences in earthworm distributions between bioregions reflected differences in cropping intensity. We thus used mixed model ANOVAs to test the effects of treatment and agricultural intensity (i.e. meadow vs. crop), as well as their interaction, on total earthworm abundances and biomass within each bioregion. These models also used site identity as a random effects variable. We subsequently used t-tests to evaluate the effects of treatment on earthworm abundance and biomass in only the sites with meadows, and only the sites with intensive cropping systems.

Finally, we used one-way mixed model ANOVAs to test the effect of dominant vegetation on total earthworm abundances and biomass in each bioregion and each treatment. Class variables in FRBS were deciduous vs. conifer vs. mixedwood, whereas those in fields were cereal vs. soy vs. meadow (Eastern Canada) or crop vs. meadows (Central Europe). These models also used site identity as a random effects variable. Separation of significantly different means was performed by Tukey HSD tests.

Distance-based redundancy analysis (dbRDA) was used to highlight correlations between vegetation types and earthworm species found in FRBS of each bioregion, using the *capscale* function in the *vegan 2.4.4* package (Oksaken et al. 2017) of R statistical software. Prior to these analyses, rare earthworm species (i.e. occurring in <10% of plots) were removed from the earthworm species composition matrix. The percent cover of bare soil and the different vegetation types were used as explanatory variables, whereas earthworm species comprised the response variables.

For each bioregion, conditional regression tree analysis was used to infer the importance of treatments and soil properties on earthworm abundances, using the *ctree* function in the *party* package of R statistical software (Hothorn et al. 2006). The response variable was log transformed after the models were generated, in order to better illustrate the data graphically. This entire procedure was repeated using earthworm biomass as well as the number of anecic, endogeic and epigeic earthworms, as response variables.

2.3 Results

In each bioregion and treatment, there were proportionately more endogeic than anecic or epigeic earthworm species (Fig. 1). For each bioregion, results from MANOVAs revealed different ($P < 0.01$) relative proportions of earthworm life habits in each treatment. More specifically, in Eastern Canada there were proportionately fewer anecic and more epigeic individuals in FRBS than in fields (Fig. 1A). In Central Europe there were fewer endogeic and more anecic individuals in FRBS than in fields (Fig. 1B).

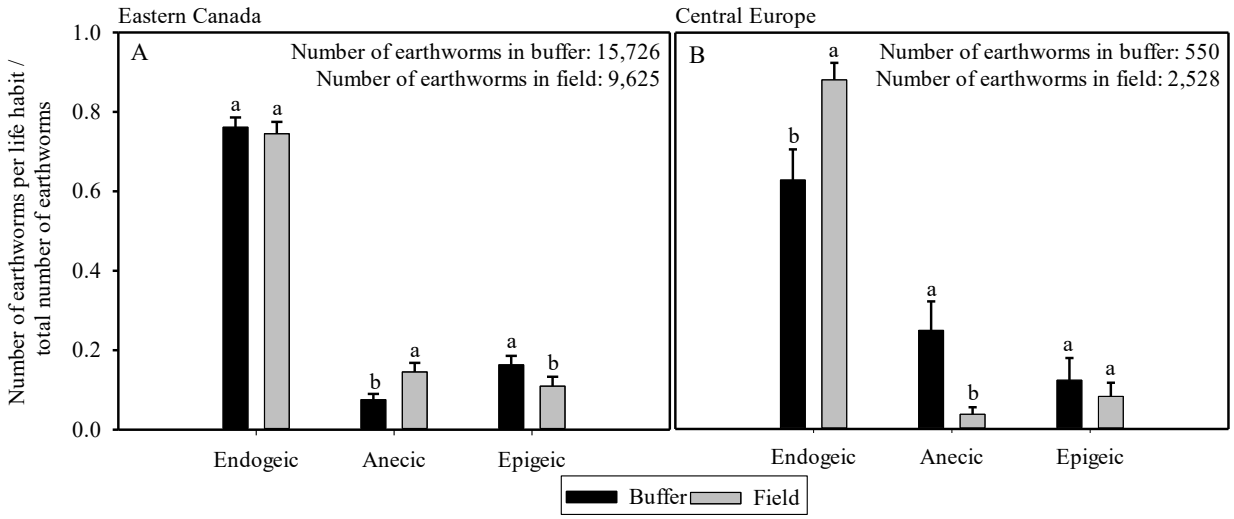

Figure 1. Proportion of earthworms by life habit in fields and buffers in (A) Eastern Canada and (B) Central Europe.

Mixed model ANOVAs revealed a significant interaction between bioregion and treatment on earthworm abundance ($P < 0.01$), earthworm biomass ($P < 0.01$) and soil moisture ($P = 0.02$) (Fig. 2). For Eastern Canada, we found significantly (Student's *t*-test, $P \leq 0.01$) higher earthworm abundance, earthworm biomass, soil moisture and organic matter in FRBS than in adjacent fields (Fig. 2A–D). For Central Europe, earthworm abundance and biomass were significantly (Student's *t*-test, $P < 0.01$) higher in fields than FRBS, whereas soil organic matter was higher (Student's *t*-test, $P \leq 0.01$) in FRBS than in fields (Fig. 2E-G). The different earthworm distributions (i.e. FRBS vs. fields) found in each bioregion were not the result of having sampled more cropped fields than meadows in Eastern Canada, and more meadows than cropped fields in Central Europe. In fact, in each bioregion we found no significant interactions between treatments and agricultural intensity (i.e. crop vs. meadows) on earthworm abundance and biomass. More specifically, the differences in mean abundance and biomass between FRBS and fields were the same in meadows as in fields, for both bioregions (data not shown).

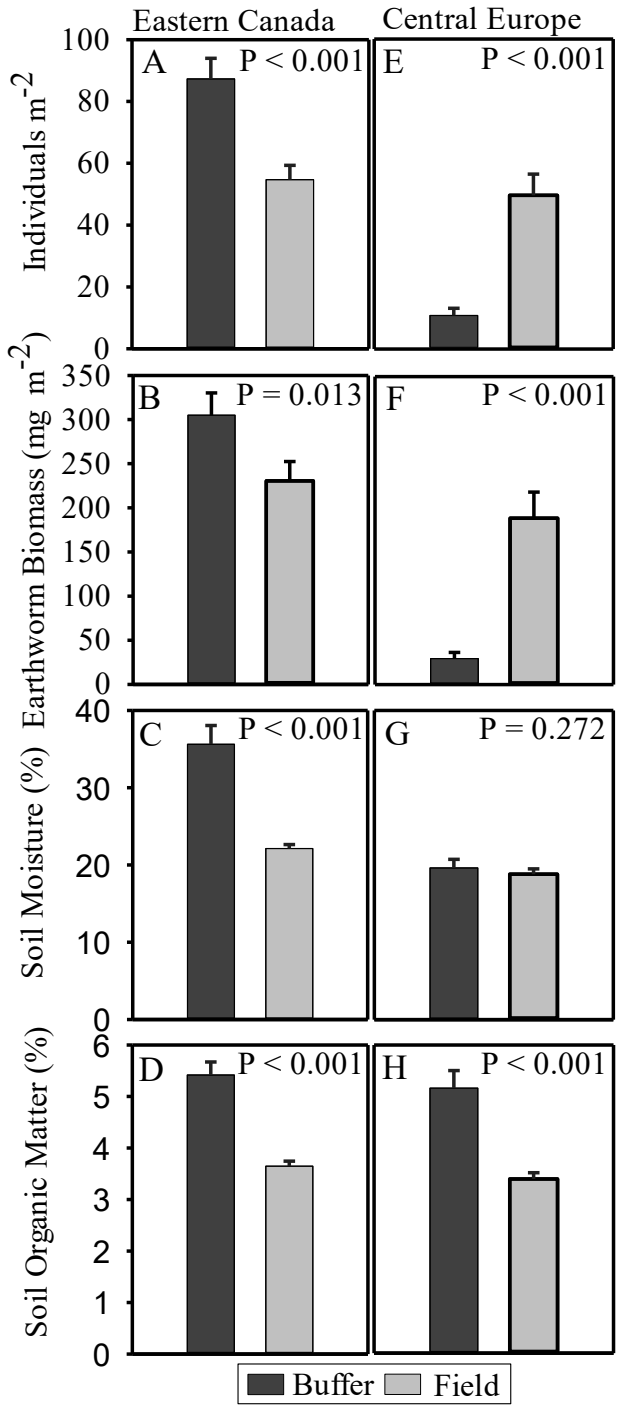

Figure 2. Comparison of earthworm numbers, soil moisture and soil organic matter in fields and buffers

In FRBS of Eastern Canada, one-way ANOVA revealed significantly higher ($P < 0.05$) earthworm abundance and biomass in deciduous and mixedwood stands than in coniferous stands (Fig. 3A, B). In FRBS of Central Europe, earthworm abundance and biomass were significantly higher ($P < 0.05$) in deciduous than in mixedwood and coniferous stands (Fig. 3C, D). In the agricultural fields of both bioregions, there was no significant effect of vegetation type on earthworm abundance and biomass (Fig. 3A–D).

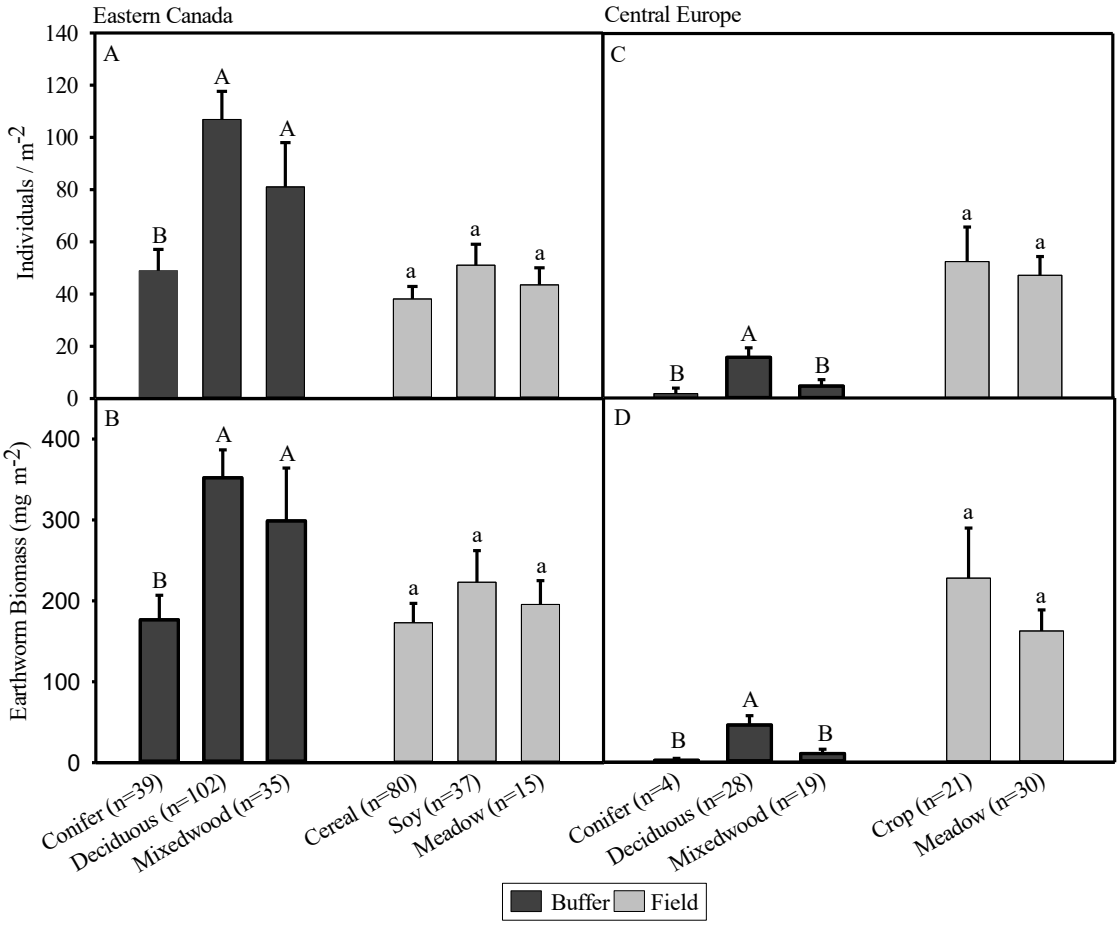

Figure 3. Earthworms abundance and biomass in different stand types in Eastern Canada and Central Europe.

Capital letters correspond to FRBS stand types and lower case correspond to agricultural field stand types.

The biplot generated from dbRDA, to correlate vegetation types and earthworm species in FRBS sampled in Eastern Canada, revealed a strong correlation of *A. rosea* with herbaceous plants along canonical axis 1 ($P < 0.01$; Fig. 4A). A similar dbRDA analysis performed on data from Central Europe revealed that the variation represented by canonical axis 1 was marginally non-significant ($P = 0.1$; Fig. 4B). Even so, we found the strongest correlation between *A. rosea* and ferns or graminoids. In both bioregions, the variation represented by canonical axis 2 was non-significant.

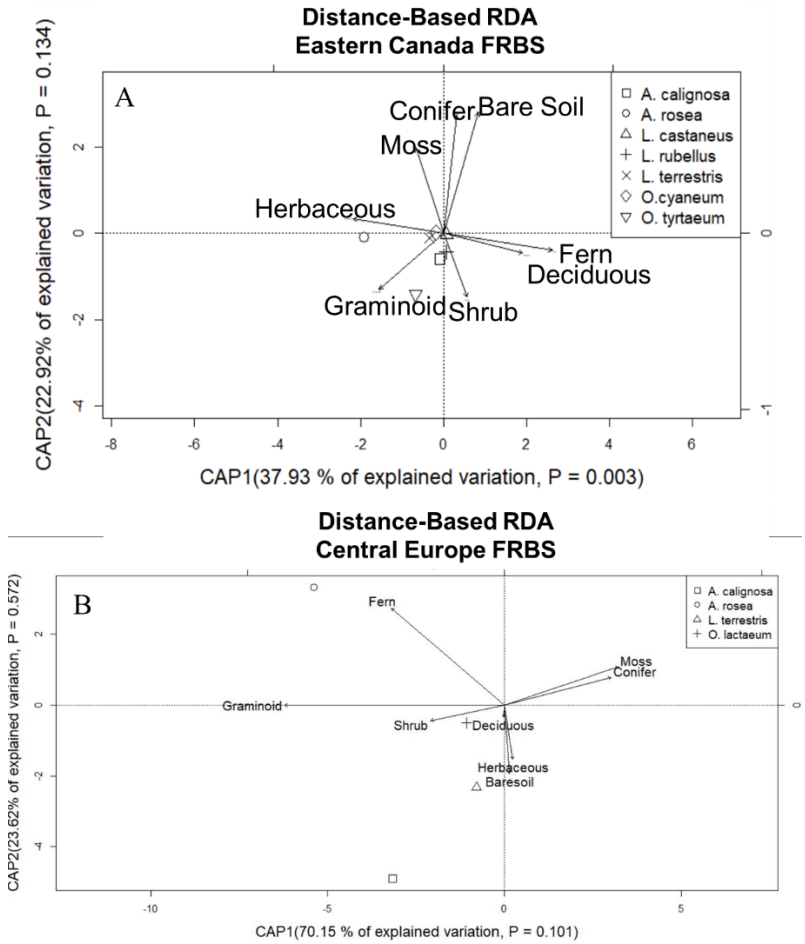

Figure 4. Results of distance-based redundancy using earthworm species as the response variables and the percent coverage of vegetation types as explanatory variables

In Eastern Canada, conditional regression tree analysis revealed that more earthworms generally occurred in sites with high (> 15%) than low (< 15%) clay content (Fig. 5). The next significant binary partition, in both high and low clay sites, was ascribed to treatments, with higher earthworm abundances in buffers than in fields (also shown in Fig. 2A-B). Further partitioning nodes in the conditional regression tree highlight the significant positive effects of pH, soil moisture and organic matter on earthworm abundances (Fig. 5). Conditional regression tree analysis in Central Europe revealed that treatments explained the most variation in earthworm abundances, with more earthworms occurring in fields than in FRBS (Fig. 6; also shown in Fig.

2E-F). Within fields, a significant node distinguished between lower earthworm abundances in sites with high (>55%) than in those with low (<55%) sand content (Fig. 6). Due to the lower total number of sites in Central Europe, the regression tree did not have the statistical power to detect further nodes. Conditional regression tree analyses for the other response variables (i.e. earthworm biomass and the number of anecic, endogeic and epigeic earthworms) did not identify any new relationships from those shown in Fig. 5 and Fig. 6.

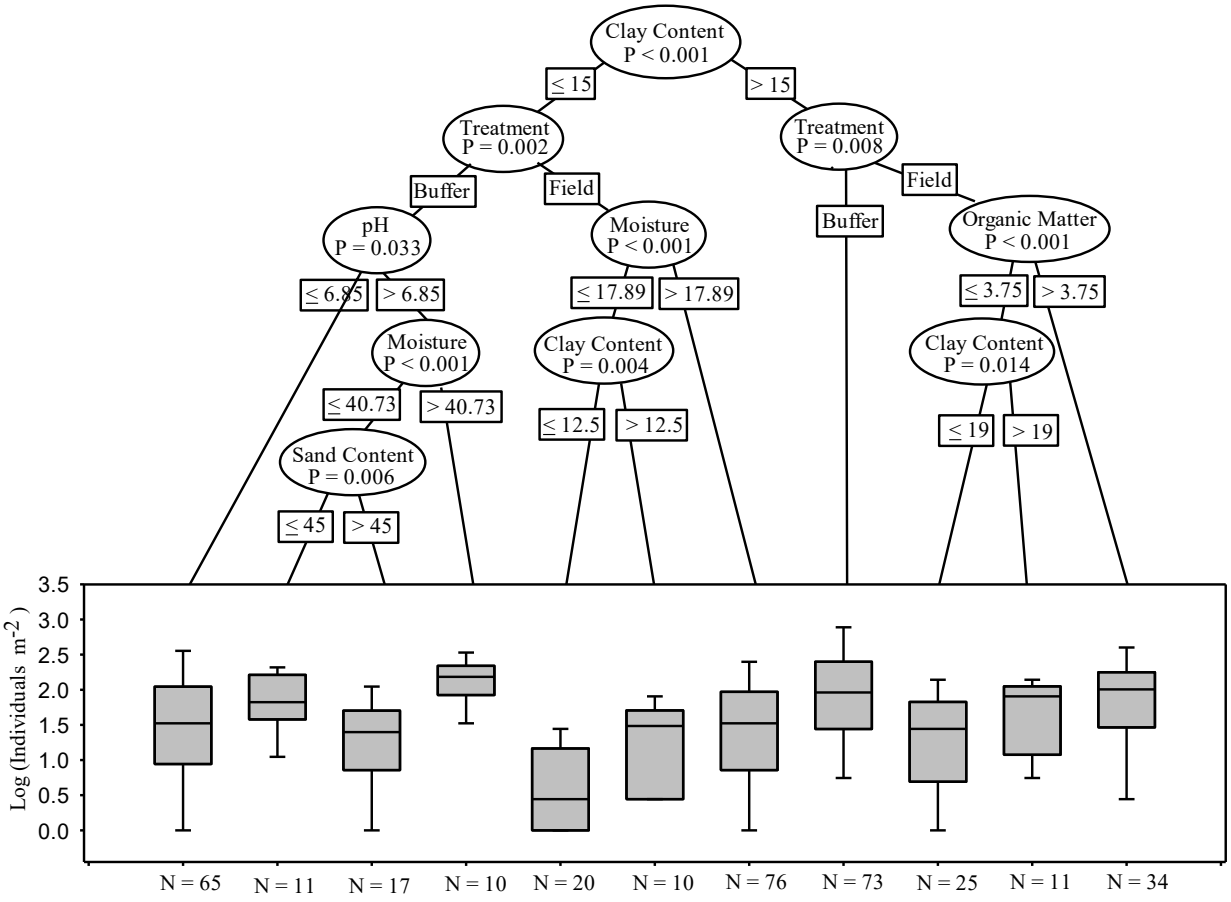

Figure 5. Results of regression tree analysis in Eastern Canada using earthworm abundance as response variable and soil and site characteristics as explanatory variables

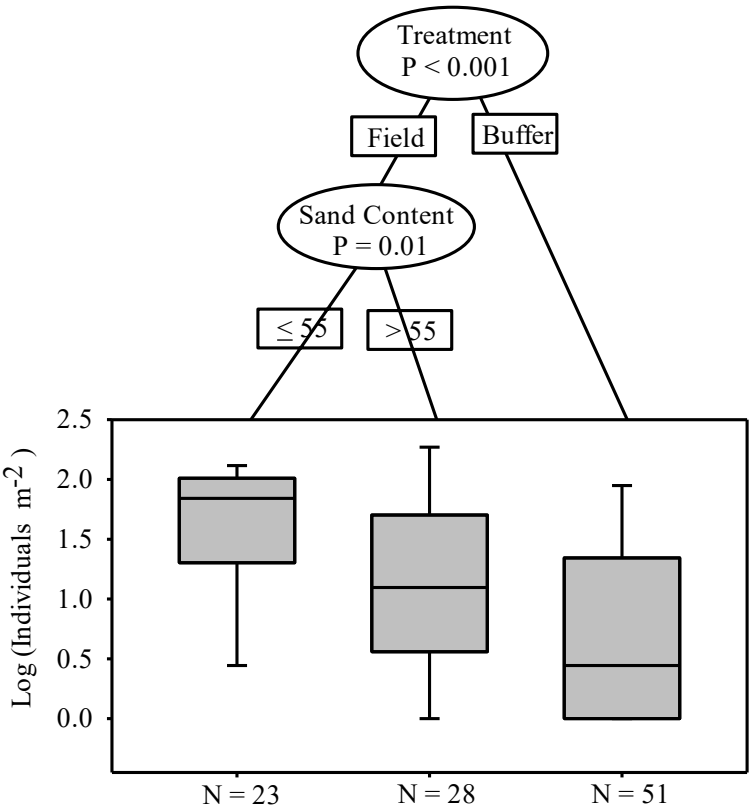

Figure 6. Results of regression tree analysis in Central Europe using earthworm abundance as response variable and soil and site characteristics as explanatory variables

2.4 Discussion

Agricultural fields and FRBS in both bioregions differed in the structure of their earthworm communities, as reflected by the relative proportion of each earthworm life habit in each of these two treatments. The relatively high proportion of endogeic species found in fields in Central Europe may reflect the higher proportion of low-disturbance meadows that we sampled in this bioregion. Endogeic species burrow horizontally and are concentrated in the rhizosphere soil (i.e. in the plow layer), and are thus likely to thrive in no tillage systems (Clapperton et al. 1997).

The greater proportion of epigeic species in FRBS of both bioregions is likely due to the presence of woody debris and stones that serve as refugia for these species (Edwards 2004). Unfortunately, we find it difficult to explain the differences we observed in the relative proportion of anecic species in each treatment. The spatial distribution of anecic earthworms also differed across bioregions, which implies that the effects of landscape features in similar ecosystems, on the structure and functional diversity of earthworm communities, can not be generalized over large continental scales. Given that earthworm community structure differed across treatments and bioregions, and given that different earthworm species differ in body mass, it was important that we verify whether earthworm abundances (i.e. individuals m^{-2}) reflect total earthworm biomass. Overall, our analyses showed that earthworm community biomass tracked very closely with earthworm abundance in all treatments and bioregions (e.g. Fig. 2A,B and Fig. 2E,F).

Perhaps our most curious result was that, contrary to what we predicted as well as contrary to what we observed in Eastern Canada, earthworm abundance in Central Europe was 5x greater in fields than in FRBS. We had initially evoked three reasons why we expected more earthworms to occur in FRBS than in fields: (1) lower soil disturbance in FRBS, (2) presumably higher soil organic matter in FRBS, and (3) presumably higher soil moisture in FRBS. Regarding the first of these hypotheses, it is true that we sampled proportionately more sites with low-disturbance meadows in Central Europe than in Eastern Canada. However, we do not believe disturbance played a major role because sites with intensive cropping and those with meadows showed a similar effect of treatments on earthworm abundances. Regarding our second hypothesis, we can also eliminate the putative role of soil organic matter quantity, given that organic matter concentrations in both bioregions were similarly higher in FRBS than in adjacent fields. As for our third hypothesis, differences in soil moisture across bioregions do provide some grounds for explaining different spatial distributions of earthworms. Our data showed that soil moisture in Eastern Canada was higher in FRBS than in fields, whereas soil moisture in Central Europe was essentially the same in both treatments. Phillips et al. (2019) explored the global drivers of earthworm diversity and found that climatic variables, namely

precipitation and temperature, were best at predicting earthworm species richness, biomass and abundance. In Central Europe, summer and early fall of 2018 were among the hottest and driest years ever recorded (Eckstein *et al*. 2019), whereas climate in Eastern Canada in 2017 and 2018 was not so anomalous. It is possible, therefore, that the summer drought of 2018 in Central Europe is the reason why soil moisture in FRBS was similar to moisture in the adjacent fields. If FRBS in Central Europe do not procure an advantage to earthworms in terms of soil moisture, we would then expect them to gravitate towards their next most suitable soil condition. From our data, we can surmise that the quantity of soil organic matter was not a determining factor. We posit, therefore, that earthworms were attracted by higher soil organic matter quality rather than quantity. For example, Szoboszlay et al. (2017) observed that the conversion of cropland to grassland increased soil bacterial abundance by a factor of 1.9, whereas conversion of grassland to forest reduced bacterial abundance by a factor of 2.3. From this information, we can infer that rhizodeposition products under agricultural crops and meadows are more abundant and/or more labile, and thus provide a more nutritious substrate for earthworms than in FRBS.

In agricultural fields, the dominant vegetation type did not have a significant effect on earthworm abundance. On the other hand, earthworm abundance in FRBS of both bioregions was significantly lower in coniferous than in deciduous stands. This was expected, as conifer litter is richer in lignin and phenolic substances, less nutritious and more acidic than litter from broadleaf trees (Reich et al. 2005). We also expect deciduous trees to have higher rhizodeposition rates than coniferous trees (Pérez-Harguindeguy et al., 2013), resulting in more nutritious rhizosphere soil. What is less clear is the effect of mixedwood stands on earthworm abundance in FRBS. In Eastern Canada, earthworm abundance in mixedwood stands was significantly higher than in coniferous stands, suggesting that pure conifer stands are needed to minimize earthworms in FRBS. In Central Europe on the other hand, earthworm abundance in mixedwood stands was significantly lower than in deciduous stands, suggesting that only a partial presence of conifers is required to minimize earthworms in FRBS.

Although the effect of overstory vegetation on earthworm abundance seems undeniable, few studies have explored the effect of forest understory vegetation. Although trees generally fix and return more carbon to the soil than forest understory plants, the percentage of fixed carbon that is transformed into labile litter and rhizodeposition products may be much higher for understory herbaceous plants than for trees. For instance, Gill and Jackson (2000) estimated that tree root systems have a 10% annual turnover rate, compared to 53% for herbaceous plants. Therefore, graminoids and other herbaceous plants are likely to be hotspots of soil organic matter quality within FRBS. Accordingly, our dbRDA analyses revealed that the strongest determinants of earthworm abundance within FRBS were herbaceous plants including graminoids. More specifically, these understory plant functional groups were strongly correlated with the earthworm species *A. rosea*, *A. caliginosa* and *O. tyrtauem*. These three species are all endogeic earthworms, which suggests that the endogeic life habit is especially favoured by the rhizosphere of herbaceous understory plants. As endogeic earthworms live exclusively belowground and feed only on mineral soil, it is logical that these species would respond the most positively to the chemical quality of the rhizosphere.

In both Eastern Canada and Central Europe, treatment had a high rank index which indicates that landscape design is important in explaining earthworm distribution. For Eastern Canada, the regression tree underscored the positive effect of moisture, pH, organic matter and clay on earthworm numbers, and the order in which they appeared in the tree highlighted their relative importance in predicting earthworm distribution in agricultural landscapes. For instance, the preference for higher organic matter was only observed in agricultural fields. This coincides with our findings that the type of vegetation in FRBS will influence earthworm numbers, therefore organic matter quantity will not be a good indicator of earthworm number across FRBS. The effect of vegetation across FRBS is also illustrated by the pH split in the tree. Coniferous trees tend to produce more acidic soils than deciduous trees, and the regression tree showed that within FRBS, earthworm numbers were higher in soils with a higher pH. In Central Europe, following the treatment split, the regression tree only underscored the negative effect that sand content had on earthworm numbers.

In summary, very few studies have been as extensive as ours and their conclusions cannot be extrapolated beyond a narrow geographical range. Conversely, our study included a large number of sites spanning a large geographical range thereby contributing to a better understanding of earthworm biogeography. A recent study (Phillips et al. 2019) looked at the global distribution of earthworms using a compilation of datasets from 56 countries. This study found that precipitation is the most important predictive variable of earthworm distribution. Our study yielded similar results since moisture and precipitation are closely related, but also added important insights into agroecosystem management. The compilation of our results allows for the design of FRBS that will either attract or repel earthworms depending on the desired outcome. For instance, if earthworms are found to counteract the positive effects of FRBS, then FRBS should be designed to deter earthworm populations, which can be achieved by planting conifers and limiting the establishment of herbaceous and graminoid plants.

2.5 Acknowledgements

We would like to thank William F.J. Parsons for providing instruction on the use of laboratory equipment and Gabriel Boilard, Anne Bolduc, Marika Caouette, Raphaëlle Dubois and Laurence Guay for assisting with the field survey and soil analyses. We would also like Naresh Thavathasn and Brent Coleman for coordinating the sampling efforts in Southern Ontario as well as Agnieszka Józefowska for providing instruction on the identification of central European earthworm species. Financial support was provided by the Agricultural Greenhouse Gas Program (AGGP) and Centre Sève, a partner of the American Phytopathological Society (APS).

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CHAPTER 3

THE EFFECT OF EARTHWORMS AND SOIL CHARACTERISTICS ON GREENHOUSE GAS EMISSIONS IN FORESTED RIPARIAN BUFFER STRIPS

This chapter reports on two microcosm experiments that tested the interacting effects of earthworms and soil characteristics on soil emissions of the three most prominent greenhouse gases (GHGs): CO_2, N_2O and CH_4. The eighteen treatments were a factorial array design with three levels of earthworm life habits (i.e. anecic, endogeic and none), soils collected from three landscape units (i.e. coniferous FRBS, deciduous FRBS and agricultural field) and two clay levels (i.e. natural and 20% enrichment). The incorporation of soil origin and soil texture in our design separates our study from other studies that aim to quantify the effect of earthworms on GHG emissions and provides the perspective required to make proposals regarding FRBS designs that minimize soil GHG emissions. Furthermore, most studies that examine the effect of earthworms on CH_4 look at net emissions, which has the potential to mask the effects of treatment on the opposing and concomitant processes of CH_4 production. Conversely, our study measured gross transformation rates, from which we can infer the mechanisms controlling net CH_4 emissions.

This paper was completed with the help of a number of co-authors. Firstly, Robert Bradley was my supervisor and assisted me throughout the two experiments in the deciding on the design, the analyses, and the writing of the manuscript. Petra Benetková constructed the microcosms and collected the soil and earthworms for the CH_4 experiment. Finally, Miloslav Šimek assisted in the development of the technique that was used for the CH_4 experiment and provided the required training, machinery and materials for the experiment. Additionally, a number of interns were involved with this project including Gabriel Boilard who assisted in collecting gas samples and running them through the GC.

Effect of earthworms and interacting soil characteristics on the emission of greenhouse gases (CO_2, N_2O and CH_4) in forested riparian buffer strips

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3.0 Abstract

Forested riparian buffer strips (FRBS) are common in temperate agroecosystems due to their ability to sequester nutrients from agricultural runoff. The full environmental benefits of FRBS can only be evaluated, however, by accounting for a wide range of criteria that go beyond stream water quality. For example, it is important to determine the net greenhouse gas (GHG) balance of FRBS relative to adjacent agricultural fields. It is also important to identify the factors controlling these GHG emissions in order to propose optimal FRBS designs that maximize their environmental benefits. One such factors is the presence of earthworms, whose burrowing activities may modify soil emission rates of CO_2, N_2O and CH_4. To test the effects of earthworms on GHG emissions, microcosm studies were conducted using a replicated factorial design comprising of 3 soil origins (deciduous FRBS, coniferous FRBS, agricultural field) x 2 soil textures (field conditions, high clay) x 3 EW life habits (anecic, endogeic, no EW). Anecic earthworms had a positive effect on soil CO_2 and denitrification, which decreased after a few weeks. Increasing soil clay content had a negative effect on the emission of these two GHGs. Additionally, soils from FRBS emitted more CO_2, N_2O and CH_4 than soils from agricultural fields.

3.1 Introduction

With the rising demand for agricultural crops, it is estimated that by 2050, 1 billon ha of land will be converted for agricultural land use (Tilman *et al.* 2011). A number of environmental impacts can be associated with this expansion, for instance, land clearing, crop production and fertilization account for approximately one quarter of greenhouse gas (GHG) emissions (Burney *et al.* 2010). This rise of GHG emissions is a topic of increasing concern with CO_2, CH_4 and N_2O concentrations increasing at annual rates of approximately 1.9%, 2% and 0.25% (Raupach *et* al. 2008, Ramussen and Khalil 1981, Wuebbles 2009). Moreover, the global warming potentials of CH_4 and N_2O are 21 and 310 times greater than that of CO_2 respectively (Nicks *et al.* 2003). As such, it will be important to identify potential GHG production hot spots in agricultural landscapes.

Forested riparian buffer strips (FRBS) are increasingly being promoted in temperate agroecosystems due their recognized ability to improve stream habitats and water quality (Parkyn et al. 2005) and their capacity to absorb nutrients from agricultural runoff (Fortier et al. 2015). However, in order to objectively assess their net environmental benefits, we must also identify the potential environmental downsides. For instance, FRBS store large amounts of carbon and are subjected to frequent wetting-drying cycles from changing water levels in the adjacent water courses providing both the anaerobic and aerobic conditions required for GHG production, thereby making them potential hotspots for soil GHG production. The production of GHG in soils is the result of a number of biotic processes: respiration for CO_2, methanogenesis for CH_4 and a combination of nitrification and denitrification for N_2O (Wrage *et al*. 2001; Kool *et al*. 2010). Understanding the factors that control these processes will be essential in assessing the GHG balance in FRBS. Earthworms have the ability to modify the soil structure and interact with microorganisms through their burrowing, casting and feeding

activities (Brown and Lavelle 2000; Lavelle *et al*. 1997). This affects the soil's substrate availability and physio-chemical properties thereby impacting the GHG production processes. Due to earthworms being prevalent in agricultural landscapes (approximately 300 and 250 mg m^{-2} in FRBS and agricultural fields respectively (Cameron 2020), their effect on soil GHG emissions will be an important consideration.

The higher earthworm abundances in FRBS are expected to increase CO_2, N_2O and CH_4 soil emissions. For instance, with respect to CO_2, earthworms accelerate the initial phase of plant litter decomposition which will increase the short-term release of CO_2 (Liu and Zou 2002). For N_2O, the earthworm gut, as well as the casts and burrows they create, provide an anaerobic microsite with favourable mineral nitrogen enrichment, available carbon and moisture levels for denitrifier activity, resulting in optimal conditions for N_2O production (Drake and Horn 2006; Elliott et al. 1991). For CH_4, the effect of earthworms remains ambiguous with some studies reporting an increase in net CH_4 (Koubova et al. 2012; Borken et al. 2000; Kamman et al. 2009) and others reporting a net decrease (Park et al. 2008; Moon et al. 2010; Kim et al. 2011). The inconsistent results between these CH_4 studies may be due to studying net rates instead of gross rates.

The consumption and production of CH_4 is mediated by redox sensitive microbial processes, which are controlled by microbial populations that are ecologically and evolutionarily distinct. Since studying net rates does not permit one to distinguish between these two processes, they can mask significant gross production and/or consumption rates (Zinder 1993; Hanson and Hanson 1996; King 1997). Consequently, to accurately assess the effect of earthworms on CH_4, one must study gross transformation rates. Furthermore, earthworms are divided into three distinct groups based on life habit meaning their effect on GHG emissions cannot be generalized.

When quantifying the effect of earthworms on CH_4, as well as on CO_2 and N_2O, one must consider that the degree and manner in which earthworms affect GHG production may be influenced by their different life habits. Of particular interest are anecic and endogeic earthworms. Endogeic earthworms live in and feed on mineral soil and the associated organic matter via horizontal burrows, whereas the larger anecic earthworms feed on litter from the surface which they incorporate into the soil via deep vertical burrows. In comparison to endogeic earthworms, anecic earthworms are expected to increase soil GHG emissions to a larger extent due to their direct interaction with the surface litter making them more effective at accelerating the initial phase of plant litter decomposition, the process by which earthworms accelerate short term CO_2 emissions. Additionally, the larger anecic earthworms would produce larger burrows and casts than endogeic earthworms, resulting in higher N_2O emissions since these two earthworm structures serve as microsites with optimal N_2O producing conditions (Drake and Horn 2006; Elliott *et al*. 1991). Similarly, the larger structures produced by anecic earthworms would have a greater impact on methanogen communities, producing more potential hotspots for CH_4 production. As such, the greater extent to which anecic earthworms modify the soil environment is expected to result in overall higher soil GHG emissions. However, the degree to which earthworms increase soil GHG emissions may be dependent on the soil characteristics of the environment they inhabit.

One such soil characteristic that may interact with earthworms in the control of soil GHG emissions is soil texture which is of particular interest since a recent study reported that clay content plays an important role in determining earthworm communities (Cameron 2020). In comparison to coarse textured soils, clayey soils have a higher capacity to hold water and organic matter (Lee 1985). Furthermore, the larger surface attractive forces of clays will increase the adhesion to organic matter, thereby facilitating the formation of aggregates. As such, soils with higher clay content would promote the ability of earthworms to induce long-term soil carbon stabilization, achieved by protecting carbon in microaggregates formed in larger macroaggregates (Bossyt et al. 2005; Pulleman et al. 2005). Increasing clay content may therefore counteract the positive effect of earthworms on soil CO_2 emissions over longer time

periods. Over shorter time periods, increased clay content is expected to slow diffusion from the soil thereby decreasing CO_2 emissions. Conversely, the processes which produce N_2O and CH_4 are limited by the presence of anaerobic conditions which is more apt to occur with clay rich soils whose pore space is already limited. As such, increasing clay content is expected to increase the positive effect of earthworms on soil N_2O and CH_4 emissions.

In order to maximize the net environmental benefits of FRBS, it will be essential to design these in such a way that minimizes soil GHG emissions. In order to determine what type of trees should be planted to achieve this goal, one must first determine how earthworms interact with the overstory vegetation, namely, how does soil origin affect the ability of earthworms to increase soil GHG emissions? The goal of our study was to determine the effect of earthworms, soil origin and soil texture on CO_2 emissions, denitrification, and gross CH_4 transformation rates. Denitrification was used to estimate N_2O emissions as it is assumed that denitrification rates are proportional to N_2O emissions (Davidson *et al*. 1986, Tiedje *et al*. 1989). Furthermore, the concentrations of NO_3 and NH_4 were studied to help explain potential denitrification as they are components along the N_2O production pathway. We hypothesized that GHG emissions from FRBS soils would be higher than from agricultural field soils due to FRBS having higher organic matter than adjacent agricultural fields as a result of continuous litter inputs while coniferous FRBS would have lower GHG emissions than deciduous FRBS due to a higher litter lignin content slowing the ability of earthworms to accelerate GHG production. Furthermore, coniferous FRBS were expected to have lower GHG emissions than deciduous FRBS due to a higher litter lignin content which will limit the earthworms' ability to accelerate the production of GHG in the soil.

3.2 Materials and Methods

This study involved two separate bioassays, both using the same experimental design (see below). The first study was performed at Université de Sherbrooke (Quebec, Canada) and measured net CO_2 emissions and estimated denitrification by the acetylene inhibition method (Davidson *et al.* 1986, Tiedje *et al*. 1989). The second study was performed at the Institute of Soil Biology (České Budějovice, Czech Republic) and measured gross transformation rates of CH_4 under both aerobic and anaerobic conditions, using an isotope dilution approach.

3.2.1 Soils, earthworms, microcosms and experimental design

The bioassays comprised of 18 replicated five times for a total of 90 microcosms. The first experimental factor, soil origin, had three levels: coniferous FRBS, deciduous FRBS and agricultural field. We collected the soil for the first bioassay from two sites in the Eastern Townships, Canada: 45°39'31.6"N 72°40'21.2"W and 45°56'21.4"N 72°51'23.0"W in September 2017. For the second bioassay, we collected soil from a site near Mlada Boleslav in the Czech Republic (50°21’26.3”N 15°01’29.7”E) in August 2018. Each of these sites consisted of an agricultural field with an adjacent FRBS having both coniferous and deciduous stands. The distance between each soil collection site was at least 50 m which is greater than the spatial variance of most soil variables (Loescher *et al.* 2014) meaning the five soil samples for each soil origin are considered distinct. We hand sifted the soil to separate any large debris and subsequently sieved the soil at 7 mm. The second experimental factor, texture, comprised of two levels: field texture and clay enriched. For the clay enriched soils, we added pure illite clay (Clay-25, Nature’s Oil, Bulk Apothecary) such that the soil’s clay content was increased by 20%. The third experimental factor, earthworm life habit, comprised of three levels: anecic, endogeic and no earthworms. We purchased anecic earthworms from local bait shops and collected endogeic earthworms in the field.

The microcosms for the first bioassay consisted of 10 cm internal diameter PVC pipes cut to a height of 15 cm which were covered with a 2 mm screen on the top and bottom. The microcosms for the second bioassays consisted of 15 cm tall screw top plastic containers with a 10 cm internal diameter and holes drilled into the lid. Each microcosm was filled with 1000 g (dry wt. equiv.) of fresh soil to which we added 2.2 g of oven-dried sugar maple litter, collected from the Morgan Arboretum in Saint-Anne-de-Bellevue, Quebec (Canada). This represents the approximate amount of annual litter fall, as estimated by Gosz *et al*. (1972).

Earthworms were added to the microcosms two weeks after the clay was added to prevent any potential adverse effects the freshly added clay may have to earthworm survival. For the first bioassay, 30 microcosms received two anecic, 30 microcosms received 6 endogeic earthworms and 30 microcosms received no earthworms. These earthworm numbers were selected in order to have the same biomass of earthworms for each earthworm treatment. The same design was applied to second bioassay with the exception of 4 or 5 endogeic earthworms being added per microcosm, and following their addition, the earthworms were left to work the soil for approximately one month prior to beginning gas analyses. The lower endogeic earthworm numbers for the second bioassay were due to a drought in the Czech Republic (Eckstein *et al.* 2019) which prevent a sufficient number of earthworms to be collected. Additionally, all of the earthworms collected were significantly below the average size. As such, it was impossible to achieve an equivalent biomass between anecic earthworm and endogeic earthworm microcosms. Therefore, at the time of gas sampling, the endogeic earthworm treatment was removed from the experimental design and presence vs. absence of earthworms was studies instead of earthworm life habit.

3.2.2 Gas sampling and analyses

3.2.2.1 CO_2 production and denitrification

We measured CO2 production from each microcosm nine times over a 10-week period by placing the microcosms in 2.3 L screw top pails equipped with rubber septa and taking a 10 mL headspace air sample after 24 hours. We analyzed the CO_2 concentration of the air samples using a Gas Chromatograph (Varian 431-GC, Walnut Creek, CA) and converted the concentration (ppm) to fluxes (nmoles g^{-1} h^{-1}) after correcting for ambient CO_2.

Because soil exposure to acetylene may bias subsequent gas measurements through experimental artifact, denitrification measurements were performed on only a subset of microcosms halfway through the bioassay (5 wk) and on the remaining microcosms on the last sampling date (10 wk). This was done by placing the microcosms in 2.3 L screw top pails and replacing 10 % v/v of the headspace with acetylene gas to prevent conversion of N_2O to N_2. After 24 hours, we took a 10 mL headspace gas sample and analyzed N_2O concentrations using a Gas Chromatograph (Varian CP-3800, Walnut Creek, CA). We converted N_2O concentrations (ppm) into fluxes (nmoles g^{-1} h^{-1}) after correcting for ambient N_2O concentrations.

3.2.2.2 Gross transformation rates of CH_4

We measured concurrent rates of CH_4 production and oxidation in soils from each microcosm, using a gaseous isotope dilution method developed by Bradley et al., 2012 under anaerobic conditions. For the aerobic assay, we filled 100 mL glass sampling bottles with 30 g soil (dry wt equiv.) of moist soil from each of the 90 microcosms and sealed these with air-tight butyl rubber stoppers. We then injected each glass bottle with 19 mL of air + 1 mL of a 99.9 atom

%^{13}C-CH_4: Air mixture (i.e. 1780 ppm CH_4 mixed in Air). This resulted in initial CH_4 concentrations of approximately 20 ppm and an overpressure of 20 mL in each glass bottle. Thirty minutes after injecting ^{13}C-CH_4 into each sampling bottle, we sampled 10 mL of headspace gas using a needle and syringe, and injected 5 mL of this gas into each of two 3 mL evacuated Exetainer vials (product code 103-828W: Labco Limited, Wycombe, U.K.). One vial from each pair was sent to the UC Davis Stable Isotope Facility (Davis, CA), where it was analyzed for its atom % ^{13}C-CH_4 abundance. The second vial was used to determine total CH_4 concentration, using a HP 5890 gas chromatograph (Hewlett Packard, Palo Alto, USA), equipped with a flame ionization detector. The glass bottles were then incubated for 3 days, in the dark and at 17 ºC, after which they were sampled a second time to determine atom % ^{13}C-CH_4 abundance and total CH_4 concentration. The anaerobic assay followed the same protocol as the aerobic assay, with the following modifications: (1) the entire atmosphere within each glass sampling bottle was replaced with Ar at the beginning of the assay, using an automated gas replacement system, as described by Bradley et al. (2012); (2) at the beginning of the assay, we injected each glass bottle with 19 mL of pure Ar + 1 mL of a 99.9 atom %^{13}C-CH_4:Ar mixture (i.e. 1780 ppm CH_4 mixed in Argon); (3) the incubation period lasted 12 days. For both the aerobic and anaerobic assays, we measured the internal pressure in each glass bottle prior to each gas measurement, using a U-shaped manometer connected to a needle. For both bioassays, we calculated the gross production rate (GPR) of CH4 using the equations outlined in Hart et al. (1994):

$$GPR = \frac{[M]_0 - [M]_t}{t} \times \frac{\log (APE_0 / APE_t)}{\log ([M]_0 / [M]_t)}$$

Where GPR = gross production rate

$[M]_0$ = $^{12+13}CH_4$ concentration at t = 30 min

$[M]_t$ = $^{12+13}CH_4$ concentration at t = number of days

t = time (days)

APE_0 = atom % excess of the labelled pool at t = 30 min

APE_0 = atom % excess of labelled pool at t = number of days

For both bioassays, we calculated the gross rates of CH_4 oxidation as the difference between the gross and net CH4 production rates.

3.2.3 Soil analyses

For both bioassays, we measured baseline soil properties prior to assembling the microcosms. We determined gravimetric soil moisture content by determining weight loss after drying fresh subsamples at 105 °C for 36 hours. We measured soil pH in water using a standard hydrogen electrode (soil:water = 1:2). We determined C and N content by gas chromatography following high temperature combustion, using a Vario Macro CN Analyser (Elementar Gmbh, Hanau, Germany). We determined percent organic matter by loss on ignition at 400 °C for 16 h. We determined soil texture using the hydrometer method (Bouyocous 1962). These results are summarized in Table 2. Additionally, for the first bioassay, we determined the initial and final NO_3^- and NH_4^+ concentrations. Soils from each of the microcosms were anaerobically incubated for 30 days. A KCL extraction was performed on each of these soils and run through an autosampler Astoria 2 (Astoria Pacifica INC, Oregon, USA).

Table 2. Physicochemical properties averaged over the five replicates for each of the three soil origins collected prior to microcosm assembly

Soil origin	Country of soil collection	Soil physiochemical properties							
		Moisture (%)	Organic matter (%)	Clay (%)	Silt (%)	Sand (%)	pH	Soil C (%)	Soil N (%)
Deciduous	Canada	16.6	7.9	16	44.2	39.8	5.8	2.7	0.22
Coniferous	Canada	14.9	7.3	16.9	38.7	44.4	5.7	2.8	0.22
Ag. Field	Canada	18.2	5.3	14.8	32.7	52.5	6.2	1.8	0.19
Deciduous	Czechia	21.7	11.0	18.5	14.3	67.2	7.9	4.9	0.44
Coniferous	Czechia	15.9	8.4	15.8	26.0	58.2	7.5	3.9	0.32
Ag. Field	Czechia	16.7	6.1	7.7	13.5	78.8	7.6	2.1	0.21

3.2.4 Statistical analyses

We used three-way ANOVAs to test the effect of the three experimental factors (soil origin, soil texture, and earthworm life habit) and their interactions on the log transformed CO_2 and N_2O fluxes, the log transformed concentrations of NO_3 and NH4, as well the log transformed CH_4 production and consumption rates under both anerobic and aerobic headspaces. For the soil origin and earthworm life habit ANOVAs, we performed Tukey HSD tests identify significant differences between experimental levels. We performed all statistical analyses using R statistical software, version 3.4.1 (R Core Team 2017).

3.3 Results

3.3.1 *CO_2 production*

The results from the ANOVA revealed that none of the interactions between experimental factors were significant throughout the entire experiment. The effects of soil origin and soil texture on CO_2 emissions were significant ($P < 0.05$) throughout the course of the experiment, while the effect of earthworms was only significant for the first seven weeks of the experiment. The Tukey HSD tests revealed that FRBS soils emitted more CO_2 than soils from the agricultural fields with deciduous FRBS soils having marginally higher emissions than coniferous FRBS soils (Fig. 7A). Clay enriched soils emitted less CO_2 than soils at field conditions (Fig. 7B). Soils with anecic earthworms emitted more CO_2 than soils with endogeic earthworms, which in turn emitted more CO_2 than soils without earthworms (Fig 7C).

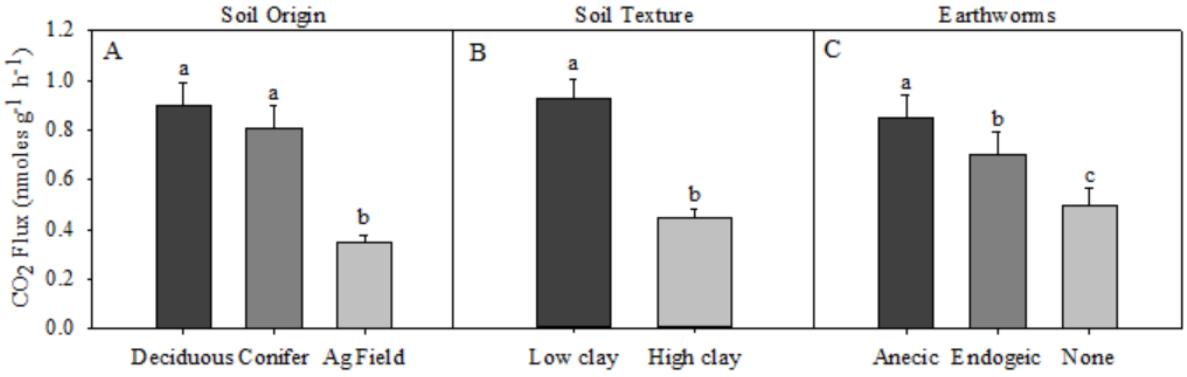

Figure 7. Mean CO_2 flux (nmoles g^{-1} h^{-1}) as affected by (A) soil origin, (B) soil texture and (C) Earthworms

3.3.2 *Nitrogen mineralization and denitrification*

The results of the three-way ANOVA revealed that earthworms did not have a significant effect on the concentration of NO_3 ($P = 0.19$), whereas soil origin and soil texture did ($P < 0.05$). High clay soils had higher NO_3 concentrations than low clay soils, and deciduous FRBS soils had higher NO_3 concentrations than field soils. The only significant experimental factor affecting NH_4 concentrations was soil origin ($P < 0.001$), whereas the effect of earthworms was marginally significant ($P = 0.06$). The Tukey HSD test revealed FRBS had higher NH_4 concentrations than field soils.

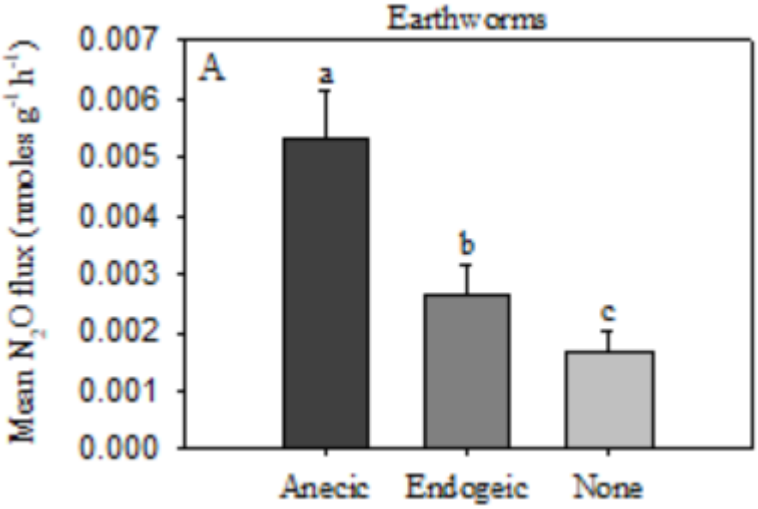

Figure 8. Mean N_2O flux (nmoles g^{-1} h^{-1}) as affected by earthworms

Results from the ANOVA revealed that earthworm life habit was the only significant ($P < 0.05$) experimental factor affecting denitrification. The Tukey HSD test revealed that soils with earthworms produced more N_2O in the presence of acetylene than soils without earthworms, and denitrification was significantly higher in soils with anecic than soils with endogeic earthworms (Fig. 8).

3.3.3 *Gross CH_4 transformation rates under aerobic and anaerobic headspaces*

The results from the three-way ANOVAs revealed that soil origin had a significant ($P < 0.001$) effect on the gross production and consumption rates CH_4 under anaerobic conditions. The Tukey HSD tests revealed that FRBS soils produced and consumed more CH_4 than field soils, but there was no difference between FRBS stand types (Fig. 9A, 9B). The results from the three-way ANOVAs revealed the significant effect of soil origin on the gross CH_4 transformation rates under aerobic conditions. The Tukey HSD tests revealed that the gross production and

consumption rates were significantly higher in deciduous FRBS soils than coniferous FRBS soils, which in turn were higher than in field soils (Fig. 9C, 9D).

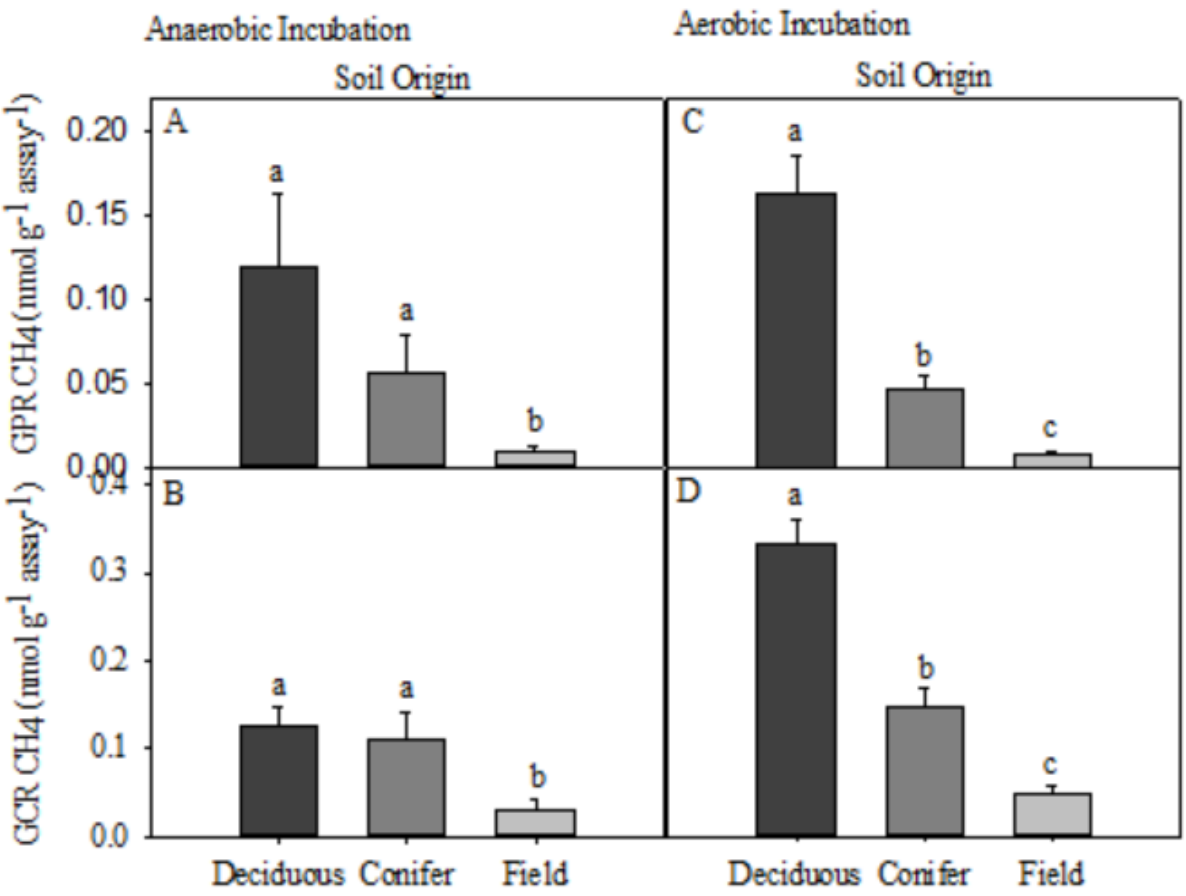

Figure 9. Mean gross production rate of CH_4 (nmoles g^{-1} h^{-1}) under anaerobic headspace as affected by (A) soil origin, mean consumption rate of CH_4 under anaerobic headspace as affected by soil origin (B), mean gross production rate of CH_4 under aerobic headspace as affected by soil origin (C) and mean gross consumption rate of CH_4 under aerobic headspace as affected by soil origin (D)

3.4 Discussion

As expected, soils with earthworms had higher CO_2 emissions, likely as a result of earthworms accelerating leaf litter decomposition by increasing litter consumption and/or digestion as well as increasing the soil's microbial activity and respiring (Liu and Zou 2002). The increased consumption of soil microbial biomass by earthworms can speed up the turnover and mineralization of microbial tissues (Araujo et al. 2003) thereby increasing the initial release of CO_2 from soils. This process also explains the higher emissions observed from anecic earthworms, which feed directly on the surface litter, in comparison to endogeic earthworms, which feed on organic matter below the soil surface (Edwards 2004). Despite this established link, earthworms were only shown to have a positive effect on CO_2 emissions during the first nine weeks of the experiment. This decrease was not due earthworm mortality since the earthworms were alive when the microcosms were deconstructed at the end of the experiment, however, it could be linked with a decrease in the labile fraction of the litter since it was not replenished. It has been suggested that earthworms may decrease CO_2 emissions by inducing long-term carbon stabilization by protecting carbon in stable microaggregates (Bossuyt and Hendrix 2005; Pulleman *et al*. 2005; Borken et al. 2000). As such, the effect of earthworms on CO_2 emissions is expected to decrease with time and the long-term storage of CO_2 by earthworms may offset their positive short-term effects (Frouz *et al.* 2014).

Soils from FRBS emitted more CO_2 than soils from fields, which is consistent with the expectation that FRBS soils have more organic matter. Moreover, CO_2 emissions were from deciduous FRBS soils than coniferous FRBS soil. This coincides with coniferous litter having a higher lignin content making the organic matter more difficult to decompose than deciduous litter (Melillo *et al*., 1982). Furthermore, the coniferous soils were slightly more acidic soils which would decompose more slowly (Prescott et al. 2000). Being that these soils decompose more slowly, coniferous FRBS are expected to promote the earthworm's ability to stabilize carbon in the soil over longer time periods.

Clay content was shown to have a negative effect on soil CO_2 emissions. It is generally accepted that the larger surface attractive forces of clays would increase the complexing of organic matter into stable aggregates. This should then increase the ability of earthworms to stabilize carbon in soils. Clay may also directly lower CO_2 emissions by slowing the rate of gas diffusion (Thorbjorn et al. 2008).

We hypothesized that soils with earthworms produced higher rates of denitrification than soils without, likely as a result of earthworms creating an environment conducive with N_2O production. Conditions for denitrifier bacteria are ideal in the earthworm gut as it provides a microsite with a local enrichment of labile C and mineral N, and also has optimal moisture conditions. These ideal N_2O producing conditions extend to soil volume that is directly influenced by earthworm activities, such mucus, casts and burrows which results in higher N_2O emissions (Drake and Horn 2006). Being larger than endogeic earthworms, anecic earthworms are expected to provide a greater volume of anaerobic microsites by creating larger soil structures and having a bigger gut, which is consistent with the observed higher N_2O emissions from soils with anecic earthworms. The concentration of NO_3 and NH_4 in the soil were also expected to impact N_2O emissions. NO_3 is an important component along the N_2O production pathway. Moreover, reducing the rate NH_4 oxidation to NO_3 during nitrification will subsequently decrease the substrate NO_3 concentration available for denitrification (Weiske *et al.* 2001; Merino *et al.* 2005). However, contrary to expectations, while soil origin and texture affected the concentration of NO_3 and NH_4 in the soil, this did not translate into a meaningful effect on denitrification.

Contrary to expectations, CH_4 production was higher under an aerobic headspace, which was likely due to differences in soil moisture which created anerobic microsites in the soil despite having an aerobic headspace. The effect of soil origin on CH_4 production under anaerobic

conditions was consistent with expectations. The production of CH_4 requires anaerobic conditions and organic matter meaning that under an anerobic headspace it stands to reason that CH_4 emissions would be higher from soils with more organic matter. Under an aerobic headspace, both the consumption and production of CH_4 responded the same way to soil origin, and neither of the other experimental factors were significant. As such, the net emissions of CH_4 are not expected to be affected by the different FRBS stand types nor the presence of earthworms.

The results from the two bioassays indicated that earthworms have a positive effect on soil GHG emissions. As such, in order to minimize net greenhouse gas emissions from FRBS in the interest of maximizing their net environmental benefits, FRBS should be designed such that they are less favourable to earthworms, meaning coniferous FRBS would be optimal over deciduous FRBS. However, this only considers the short-term effects of earthworms on soil GHG emissions. The potential of earthworms to decrease long-term emissions may offset their positive effects, therefore, further studies with a longer experimental period are required to understand the full scope of the effect of earthworms on soil GHG emissions.

3.5 Acknowledgements

We would like to thank William F.J. Parsons for providing instruction on the use of laboratory equipment, Gabriel Boilard for assisting the collection of GHG samples and running them through the gas chromatographs as well as Marika Caouette and Laurence Guay for assisting with gas analyses for CO_2 and N_2O. Financial support was provided by the Agricultural Greenhouse Gas Program (AGGP) and Centre Sève, a partner of the American Phytopathological Society (APS).

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CHAPTER 4

GENERAL DISCUSSION AND CONCLUSION

Earthworms with different life habits will interact with the soil environment in different ways. Furthermore, their different feeding habits will affect their preferences for environmental characteristics. As such, earthworm numbers alone will not be sufficient in reaching meaningful conclusions with these studies. Earthworm life habits will also need to be studied both in determining the environmental factors driving earthworm distribution as well as in determining the earthworm effect on FRBS soil GHG emissions. Consequently, earthworm biomass was added as a response variable in the study of earthworm distribution in agroecosystems since this will take into consideration the difference in sizes between the different life habits. Additionally, two earthworm life habits were used when assessing the effect of earthworms on soil GHG emissions instead of simply comparing presence and absence. In looking at the global objective of determining the net soil GHG emissions of FRBS as affected by earthworms, the first step was to determine which environments will be conducive with high earthworm numbers and subsequently determine how these environments affect soil GHG emissions.

Although FRBS were expected to serve as a refuge for earthworms in agroecosystems due to higher organic matter and soil moisture, this hypothesis was only true for Eastern Canada. In both bioregions, organic matter was higher in FRBS than fields whereas moisture was the same in FRBS and fields in Central Europe, likely as a result of the drought in 2018. As such, organic matter does not appear to be as significant in explaining earthworm distribution as soil moisture. Earthworms are more affected by carbon fluxes in their immediate vicinity (Dindal et al 1977) and therefore do not benefit from the large amounts of organic matter produced by trees. As

such, earthworms are responding more to organic matter quality than organic matter quantity (Lee, 1985; Edwards and Bohlen, 1996).

The availability of nutrients in the rhizosphere is controlled by the release of organic substances from roots which will be influenced by vegetation since the quality and quantity of root exudates is related to plant species. As such, while coniferous and deciduous FRBS will have more organic matter than adjacent agricultural fields, they will differ in the availability of organic matter. Pérez-Harguindeguy et al. (2013) found that high specific leaf area is linked to high growth rate resulting in larger amounts of exuded carbon. Consequently, deciduous stands would have higher root exudation than coniferous stands making deciduous FRBS more favourable to earthworms. Furthermore, this higher turnover increases the rate of decomposition which explains the higher CO_2 emissions from deciduous than coniferous soils. This was also true for CH_4 emissions under aerobic conditions where deciduous FRBS emissions were higher than coniferous FRBS emissions likely due the higher abundance of more readily available organic matter which is a limiting component in CH_4 production. Unlike the other two GHG gases, N_2O emissions was not affect by organic matter quality or quantity, which is contrary to expectations as N_2O production is limited by organic matter availability.

The aforementioned preference for deciduous FRBS was expected to explain the difference in earthworm distribution between Eastern Canada and Central Europe in that if Central Europe had a higher proportion of coniferous FRBS than deciduous FRBS it could explain the preference for agricultural fields. However, this was not the case as the proportion of coniferous stands was lower in Central Europe than in Eastern Canada. Additionally, the higher proportion of agricultural meadows in comparison to intensive agriculture fields in Central Europe is more likely to explain the preference for fields than the different stand types. Lee et al. (2008) found that soils with grasses had higher microbial activity than soils without. Consequently, soils which are dominated by grasses are expected to provide more readily available organic matter for earthworms. As such, when moisture is the same, as was the case between fields and FRBS

in Central Europe, earthworms will prefer an environment with higher graminoid cover, namely fields. Additionally, within FRBS, understory vegetation will play an important role in determining earthworm numbers.

As expected, earthworm numbers were higher in deciduous FRBS than coniferous FRBS. However, the relationship was not linear and earthworm numbers were shown to respond more to understory vegetation. As previously outlined, earthworms are responding more to the availability of organic matter which will be controlled by fine root turnover. The lifespan of fine roots is relatively short resulting in a more rapid turnover rate of carbon and nutrients from root mortality in comparison to larger roots (Pregitzer et al. 2002). For instance, Gill and Jackson (2000) found that entire tree root systems had a slower turnover rate (10% annually) compared to grassland which had a quicker turnover rate (53 % annually). As such, higher herbaceous and graminoid plant cover would result in more readily available organic matter in the rhizosphere creating an optimal environment for earthworms. This will be especially true for endogeic earthworms which live in and feed on the mineral soil and will therefore be more affected by rhizosphere quality. In addition to vegetation, soil characteristics were expected to affect earthworm distribution.

As already outlined, earthworms were shown to prefer higher moisture and organic matter. Additionally, in Eastern Canada, earthworms also preferred soils with a higher pH. This reinforces the observed preference for deciduous stands over coniferous stands as coniferous trees tend to produce more acidic soils. This will also have an implication on soil GHG emissions as acidic soils tend to decomposed more slowly (Prescott et al. 2000) which would slow the production and emission of soil GHG. Furthermore, in both Eastern Canada and Central Europe earthworms preferred clay rich soils over sandier soils. Firstly, the coarser sand particles would be more abrasive and cause damage to earthworm skin (Williamson 2004) thereby elucidating a preference for clay rich soils. Additionally, coarser soils such as sands have a lower

capacity to hold water and organic matter in comparison to fine textured soils such as clays (Lee 1985). Therefore, due to the well-established preference for high soil organic matter and moisture, clay rich soils would produce an environment that is optimal for earthworms. Furthermore, soil texture has the ability to affect soil GHG emissions. The emission of CO_2 was lower from soils having a higher clay content. Having larger surface attractive forces, clays would increase adhesion to organic matter therefore facilitating the protection of carbon into aggregates and reducing long-term CO_2 emissions (Bossyt et al. 2005; Pulleman et al. 2005). Over shorter time periods, low CO_2 emissions from clay enriched soils are likely the result of a slower gas diffusion rate from finer textured soils as identified by Thorbjorn et al. (2008). However, this relationship was not observed for N_2O and CH_4. Knowing which factors explain the distribution of earthworms in agroecosystems and how these environmental characteristics affect soil GHG emissions, the next step is to determine the role of earthworms in the control of each of the three GHGs.

As expected, earthworms increased the initial soil CO_2 emissions with higher emissions from soils with anecic earthworms than soils with endogeic earthworms. This is likely the result of earthworms increasing the rate of leaf litter decomposition by increasing soil microbial activity (Liu and Zou 2002). The direct connection with leaf litter explains the higher emissions from anecics which feed directly on the surface litter unlike endogeics which feed on materials in the mineral soil (Edwards 2004). However, earthworms only increased CO_2 emissions during the first 9 weeks of the experiment. This could be the result of earthworms inducing long-term carbon stabilization in the soil by protecting carbon in stable microaggregates (Bossuyt and Hendrix 2005; Pulleman *et al*. 2005; Borken et al. 2000). Consequently, the effect of earthworms on CO_2 emissions is expected to decrease with time.

As was the case with CO_2, soils with earthworms emitted more N_2O than soils without and emissions were higher from soils with anecic earthworms than soils with endogeic earthworms.

This likely due to the earthworms producing an optimal environment for N_2O production. Earthworms will provide a continuous source of labile carbon and mineral nitrogen. Furthermore, their guts, as well as the structures they create in the soil volume, provide the anaerobic environments required for N_2O production (Drake and Horn 2006). Since anecic earthworms are larger than endogeics, their structures and guts are also large thereby creating a larger volume of environments conducive with N_2O production. Unlike the two previous GHG, earthworms did not have a positive effect on CH_4 emissions.

Although the presence of earthworms did not increase soil emissions for all three GHG, the overall effect was positive. Furthermore, their positive effect on N_2O emissions is of particular concern since the warming potential of N_2O is 310 times that of CO_2. Therefore, in the interest of maximizing the environmental benefits of FRBS, they should be designed to prevent earthworm establishment. Based on the results from the field survey, this can be achieved by planting coniferous trees instead of deciduous. Firstly, coniferous trees were shown to have a negative effect on earthworm establishment. Secondly, in the event that earthworm populations do become established, coniferous FRBS soils tended to have lower GHG emissions than deciduous FRBS soils. Furthermore, due to its positive effect on earthworm numbers, herbaceous plant cover should be limited in FRBS.

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